Saskatchewan Agriculture, Food and Rural Revitalization

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

20000242

PEST STATUS OF LEAFHOPPERS AND THEIR IMPACT ON ASTER YELLOWS DISEASE IN CANOLA

Funded by: The Agriculture Development Fund

April, 2004

Prepared by: Receiver General for Canada

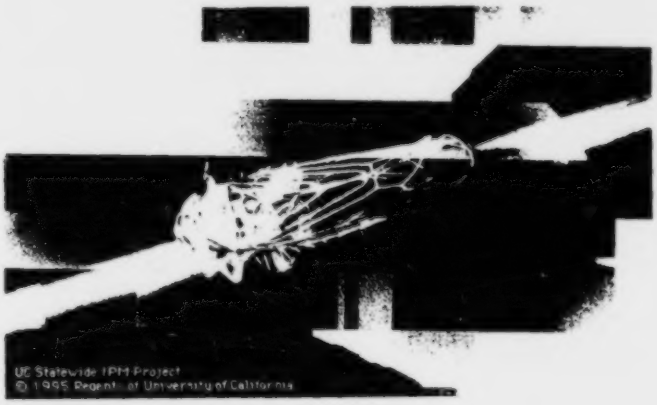
105-00314/

Saskatchewan Agriculture Development Fund

Final Report

Project #20000242

Pest status of leafhoppers and their impact on aster yellows disease in canola



O.O. Olfert, G. Séguin-Swartz, C.Y. Olivier

AAFC – Saskatoon Research Centre
107 Science Place, Saskatoon, SK S7N 0X2

March 15, 2004

Final Report to the Saskatchewan Agriculture Development Fund

Date: March 15, 2004

Reporting Period: January 1, 2001 to December 31, 2003

Principal Investigator: Dr. O.O. Olfert

Co-investigator: Dr. G. Séguin-Swartz

Project Number: 20000242

Project Title: Pest status of leafhoppers and their impact on aster yellows disease in canola

EXECUTIVE SUMMARY

Phytoplasmas are associated with yellows diseases in several hundred plant species (McCoy et al. 1989). The organisms have been classified in 20 major phylogenetic groups, of which the aster yellows group is the largest (Seemüller et al. 1998). In Canada, aster yellows diseases can be found in a variety of field and horticultural crops such as canola, alfalfa, vegetables, herbs and spices and in shrubs such as saskatoon berries and raspberries, and trees of agricultural importance (Griffith et al. 1999). Aster yellows disease in canola has been reported to be caused by phytoplasmas belonging to strains 16SrI-A and 16SrI-B (Wang and Hiruki 2001).

The transmission of phytoplasmas to plants generally occurs via phloem-feeding insects, primarily leafhoppers. Other known vectors include species of planthoppers (Cixiidae) (Weber and Maixner 1998), psyllids (Psyllidae) (Davis and Eyre 1996), froghoppers (Cercopidae) (Tsai 1979) and stinkbugs (Pentatomidae) (Hiruki 1998). On the Canadian prairies, the six-spotted aster leafhopper *Macrosteles quadrilineatus* Forbes (formerly *M. fascifrons* Stål) is presumed to be the predominant vector for aster yellows (Chiykowski 1965). It has been reported that infestations of *M. quadrilineatus* can result from local resident populations emerging from overwintering eggs and from insects migrating on favourable winds from the southern USA (Chiykowski and Chapman 1965).

Other species of leafhoppers commonly found on the Canadian prairies are known to be phytoplasma vectors. For instance, *Neokolla hieroglyphica* Say transmits the alfalfa witches' broom (Khadhair et al. 1997), *Scaphytopius acutus* Say transmits both the California aster yellows and Western X diseases (Tsai 1979). Species of the genus *Euscelis* transmit cabbage witches' broom, rape phyllody and clover phyllody (Lee et al. 1998). *Endria inimica* Say and species of *Gyponana* transmit the aster yellows phytoplasma (Hill and Sinclair 2000, Beanland and Wolf 2003). Species of *Ceratagalia* are known to be phytoplasma carriers (Khadhair et al. 1997). These species need to be considered as potential vectors of aster yellows in canola, as some leafhoppers can transmit several strains of phytoplasmas (Tsai 1979, Lee et al. 1998).

In the course of the project, we have found that *M. quadrilineatus* can overwinter not only as eggs in leaf tissue of winter grains as previously reported (Chiykowski and Chapman 1965, Chiykowski 1981), but also as adult insects in the leaf litter. No infected overwintering adults were detected in the study. High numbers of overwintering adults of the phytoplasma carrier *C. humilis* were trapped in the spring near Medstead and Saskatoon and phytoplasmas were detected in these overwintering adults.

In 2001 and 2002, only adults of *M. quadrilineatus* were found in canola crops while large numbers of adults and nymphs were found in cereals and herb and spice crops, suggesting that these leafhoppers are not reproducing in canola crops. On average, the number of aster leafhoppers in cereal fields was three to seven times the number of aster leafhoppers observed in canola fields. Depending on the climatic conditions, two to three generations of *M. quadrilineatus* occurred during the growing season in Saskatchewan.

PCR analysis revealed that *M. quadrilineatus* collected in canola, cereal, and herb and spice crops were infected with phytoplasmas, as were several other leafhopper species. In addition to *M. quadrilineatus*, we detected phytoplasma DNA in *C. humilis*, *A. inimicus*, *N. hieroglyphica*, *S. acutus*, *Euscelis* sp., *Gyponana* sp. and *Psammotettix* sp. Phytoplasma DNA was also found in two species not known to carry or to vector phytoplasmas, namely, *Diplocolenus configuratus* Uhler and *Sorhoanus ulheri* Oman.

RFLP analysis performed on individuals of *M. quadrilineatus* indicated that the insects contained the aster yellows phytoplasma belonging to strain 16SrI-B, while individuals of *C. humilis* contained phytoplasmas belonging to strain 16SrI-A or strain 16SrI-B.

In the current project, aster yellows phytoplasmas belonging to strains 16SrI-A and 16SrI-B were detected in leaf tissue, as reported by Wang and Hiruki (2001), and in root and stem tissue as well as in seed of symptomatic canola plants. The detection of the aster yellows phytoplasma in canola seed is a novel result and raises the issue of potential seed transmission of the disease.

In addition, aster yellows phytoplasmas were detected in tissue of canola plants showing atypical symptoms (entirely or partially purple main stem bearing normal siliques containing well-formed and small, misshapen seeds). These symptoms need to be added to the list of aster yellows symptoms noted during disease surveys.

The development of colonies of healthy and infected *M. quadrilineatus* for use in resistance screening was undertaken. A colony of healthy leafhoppers was established and has been maintained successfully since 2001. The development of a colony of phytoplasma-infected leafhoppers was undertaken but the attempts to obtain infected leafhoppers by placing healthy insects in contact with phytoplasma-infected plants were unsuccessful. Further research is required to develop a colony of phytoplasma-infected insects for resistance screening.

The study also led to the establishment of the complete life cycle of the aster yellows disease in Saskatchewan, which was published in Diseases of Field Crops in Canada (Bailey et al. 2003).

TABLE OF CONTENTS

EXECUTIVE SUMMARY 2

INTRODUCTION 6

PART 1 – Identification of the phytoplasma and the insect vector 6
1.1 Materials and Methods 7
1.2 Results 7
1.3 Discussion 8

PART 2 – Epidemiology of the aster yellows disease in canola crops 9
2.1 Overwintering of infected and non-infected leafhopper populations 9
2.1.1 Materials and Methods 10
2.1.1.1 Emergence traps 10
2.1.1.2 Pitfalls 10
2.1.1.3 Suction trap 10
2.1.1.4 Sweeping nets 11
2.1.2 Results 11
2.1.3 Discussion 12
2.2 Leafhopper population survey during the growing season 13
2.2.1 Materials and Methods 13
2.2.2 Results 14
2.2.3 Discussion 15
2.3 Aster yellows disease survey 15
2.3.1 Materials and Methods 16
2.3.2 Results 16
2.3.3 Discussion 17

PART 3 – Aster yellows germplasm screening 18
3.1 Leafhopper rearing 18
3.2 Germplasm screening 18

PART 4 – Transmission of phytoplasmas 19
4.1 *Brassica napus* canola 19

4.2 *Brassica rapa* canola..... 19

LITERATURE CITED..... 21

PROJECT DEVELOPED MATERIALS 25

ACKNOWLEDGEMENTS 27

TABLES

FIGURES

PEST STATUS OF LEAFHOPPERS AND THEIR IMPACT ON ASTER YELLOWS DISEASE IN CANOLA

INTRODUCTION

In Canada, aster yellows disease affects several economically important crops including oilseed and vegetable crops, herbs, spices, forage species, shrubs and many tree species. Aster yellows are caused by phytoplasmas, which are plant pathogenic mollicutes related to the Acholeplasmas. Aster yellows phytoplasmas are transmitted by phloem-feeding insects, mainly leafhoppers. In canola (*Brassica napus* L.), the aster yellows phytoplasma is presumed to be transmitted by the six-spotted leafhopper, *Macrosteles quadrilineatus* Forbes.

The disease has been reported in Manitoba and Saskatchewan in most years since 1953 and, with the exception of an epidemic in 1957 (Vanterpool 1963), the disease has been considered to be of little importance, with incidences lower than 1% infected plants. The incidence of aster yellows in canola crops on the Canadian prairies increased substantially from 1997 to 2000 (Pearse et al. 2000, Benard et al. 2001) and a project was undertaken to examine the underpinning causes. The objectives of the project were to identify the phytoplasma and the insect vectors, to determine the epidemiology and impact of the disease in canola and to attempt to screen canola germplasm for resistance/tolerance to aster yellows. The study was designed to generate information required for the development of technologies for early-warning systems and pest management strategies.

PART 1 – Identification of the phytoplasma and the insect vector

The first part of the research was concerned with the identification of the phytoplasma(s) implicated in aster yellows disease in canola. Fields near Medstead, North Battleford, Turtleford, Meadow Lake, Lanigan, Brooksby, Kelvington, Canora, Coderre and Vonda were visited on several occasions each year as well as a site at the AAFC-Saskatoon Experimental Farm (Table 1). Other fields were visited in particular years (Table 1). Insects were collected with emergence traps, pitfalls, sweeping nets, a suction trap and sticky traps, as described in Sections 2.1.1 and 2.2.1.

The various insect and plant samples collected in the course of the study were stored at -20°C until they were examined for the presence of phytoplasma DNA. Detection of phytoplasma DNA was performed using PCR (Polymerase Chain Reaction) technology in the laboratory of Dr. V. Ripley, AAFC-Saskatoon Research Centre. The identification of the phytoplasma strain was performed by RFLP analysis in the laboratory of Dr. E. Boudon-Padieu, Unité Mixte de Recherche 1088 Plante Microbe Environnement, INRA-Université de Bourgogne, Dijon, France.

Insects collected in 2001 were analysed individually. In 2002 and 2003, insects of species that had tested positive for phytoplasma DNA in 2001 were again examined individually while insects of species that had tested negative for the presence of phytoplasma DNA were analysed by groups of 10 or 20 individuals, depending of the size of the insects. Leaf, stem and root tissue and seeds of collected plants were tested for the presence of phytoplasma DNA using PCR technology. RFLP analysis was performed on insect and plant samples harvested in 2001.

1.1 Materials and Methods

DNA was extracted according to Daire et al. (1997) from individual insects, or groups of insects, and from approximately 1 g fresh weight of leaf, root and stem tissue and from bulked samples of five to 10 seeds per plant sample. DNA was amplified by nested PCR using a modified protocol of the technique described by Tanne et al. (2001). Nested PCR was performed using two "universal" phytoplasma-specific primer sets, primer pair P1/P7 for the first amplification and primer pair U5/U3 for the second amplification. Since the primers are universal, the 860-bp product obtained with nested PCR indicates the presence of phytoplasma DNA but does not specifically detect DNA of the aster yellows phytoplasma.

PCR amplification was performed using an automated thermal cycler (PRC-220, DNA engine Dyad, MJ Research, Inc., San Francisco, California, USA), with Taq polymerase (New England Biolab, Beverly, California, USA) at 1 unit/20 µl. The first and second amplifications were performed in 20 µl mixtures containing 2 µl of 10X Invitrogen buffer (Invitrogen Canada Inc., Burlington, Ontario, Canada), 200 µM each dNTP, 0.5 µM each primer and for the first amplification, 4 µl of undiluted DNA with 5 mM $MgCl_2$ and for the second amplication, 2 µl of diluted PCR product (1/1000) of the first amplification with 2.5 mM $MgCl_2$. For the first amplification, denaturation was performed at 92°C for 1 min 30 s, followed by 30 cycles of 45 s at 92°C, 45 s at 57°C and 1 min 45 s at 72°C. For the second amplification, denaturation was performed at 92°C for 1 min 15 s, followed by 35 cycles of 30 s at 92°C, 30 s at 67.5°C and 1 min 15 s at 72°C. The PCR products were separated by electrophoresis on 2% agarose gels followed by staining in ethidium bromide (0.5 µg/ml) and visualization of the DNA bands with a UV transilluminator. As stated above, the presence of a 860-bp product in the gels confirmed the presence of phytoplasma DNA in the extracts.

RFLP analysis was performed according to Griffith et al. (1999). Briefly, aliquots of PCR products were digested overnight at 37°C with the enzymes *Alu*I or *Tru*9I. Restriction digest products were separated by electrophoresis in 10% acrylamide gels prepared in TBE (Tris/borate/EDTA) and were stained with ethidium bromide (0.5 µg/ml). DNA bands were visualized with a UV transilluminator. RFLP analysis was performed on 30 leaf and stem samples of canola plants that presented aster yellows symptoms and 25 adults of *M. quadrilineatus* (caught near Kelvington) and five adults of *C. humilis* (caught near Medstead) that tested positive for the presence of phytoplasma DNA.

1.2 Results

Ten leafhopper species tested positive for the presence of phytoplasma DNA at least once over the course of the study. The species were (Figures 1-3):

Macrosteles quadrilineatus Forbes
Ceratagalia humilis Kirk.
Er lria inimica Say
Gyponana sp.
Neokolla hieroglyphica Say
Psammotettix sp.
Scaphytopius acutus Say
Euscelis maculipennis Delong & Davidson

Diplocolenus configuratus Uhler
Sorhoanus ulheri Oman

The identification of the insects that were found to be positive for the presence of phytoplasma DNA was subsequently confirmed by Dr. A. Hamilton, AAFC- Eastern Cereal and Oilseed Research Centre, Ottawa.

The first seven species are known to be phytoplasma carriers or vectors (Table 2). *Macrosteles quadrilineatus* showed the highest number of infected leafhoppers, followed by *C. humilis* (Table 3). The percentage of infection was similar for *M. quadrilineatus* and *C. humilis* in 2001 and 2003. The number of infected *E. inimica* and *D. configuratus* was low but constant every year. The other species were found to be infected occasionally.

RFLP analysis determined that all adult insects of *M. quadrilineatus* were infected with strain 16SrI-B (commonly known as the California aster yellows phytoplasma), while four adult insects of *C. humilis* were infected with strain 16SrI-A (commonly known as the Eastern aster yellows phytoplasma) and one adult specimen of *C. humilis* was infected with both strains.

Plants harvested near Kelvington were infected with aster yellows phytoplasma strain 16SrI-B while plants harvested near Togo were infected by either strain 16SrI-A or strain 16SrI-B.

Mixed infection, i.e., both strains occurring in the same sample, was not detected in any of the insect and plants samples that were examined.

1.3 Discussion

The high numbers of infected adults of *M. quadrilineatus* found in most crops indicate that this species should be considered to be the main vector for the aster yellows phytoplasma. The species is also likely to transmit the phytoplasma to plants that act as phytoplasma reservoirs. The specimens of *M. quadrilineatus* were infected only by strain 16SrI-B; *M. quadrilineatus* has been reported to vector both strain 16SrI-A and 16SrI-B in the USA (Lee et al. 2003).

Ceratagalia humilis showed lower numbers of infected insects, but its frequency of infection was similar to that of *M. quadrilineatus*, placing *C. humilis* at the second rank for potential phytoplasma vector. Further research is required to verify that *C. humilis* can vector the aster yellows phytoplasma. *Ceratagalia humilis* is mainly found at the edges of crop fields and in pastures and glades, and overwinters as adults and nymphs under the leaf litter (Beirne 1957). This suggests that *C. humilis* can be a potential vector of the phytoplasma to perennial plants that act as phytoplasma reservoirs. RFLP analysis revealed that specimens of *C. humilis* were infected with strain 16SrI-A or 16SrI-B, as observed in the United States (Lee et al. 2003).

Low numbers of infected adults of *E. inimica*, a species known to be an aster yellows phytoplasma vector, were found each year, indicating that *E. inimica* should be considered as part of the aster yellows phytoplasma vector complex.

Very low numbers of infected and non-infected adults of *Gyponana* sp., *N. hieroglyphica*, *Psammotettix* sp. and *S. acutus*, species known to be phytoplasma vectors, were found in most crops. This suggests that these species do not play a major role in spreading the aster yellows disease in canola and cereal crops in Saskatchewan.

The identification of the phytoplasma strain carried by infected adults of *E. inimica*, *Gyponana* sp., *Psammotettix* sp. and *S. acutus* remains to be performed to determine whether these species play a role in spreading the aster yellows disease to canola and phytoplasma reservoir species.

Low numbers of infected adults of *S. ulheri* and *D. configuratus* were found every year. To our knowledge, it is the first time that *D. configuratus* and *S. ulheri* have been found to be positive for phytoplasma DNA. The phytoplasma strain present in these species has yet to be identified to determine whether the species can vector the aster yellows phytoplasma.

The research determined that canola plants can be infected by both aster yellows strains 16SrI-A and 16SrI-B, as reported by Wang and Hiruki (2001). Plants infected with either strain were collected near Togo while plants infected with only strain 16SrI-B were collected near Kelvington, suggesting a different geographical distribution of the aster yellows phytoplasma in Saskatchewan.

In summary, in Saskatchewan, the aster yellows phytoplasma vector complex consists of:

- *Macrosteles quadrilineatus* (major vector species)
- *Ceratagalia humilis* (potentially the second most important vector species)
- *Endria inimica* (found in low numbers, minor vector species)
- *Diplocolenus configuratus* and *Sorhoanus ulheri* (found in very low numbers, potentially minor vector species)

The aster yellows strain present in the phytoplasma vector *M. quadrilineatus* is 16SrI-B (California aster yellows phytoplasma). The aster yellows strain present in the phytoplasma carrier (and potentially phytoplasma vector) *C. humilis* is either 16SrI-A (Eastern aster yellows phytoplasma) or 16SrI-B.

Both aster yellows strains 16SrI-A and 16SrI-B are present in infected canola plants.

PART 2 – Epidemiology of the aster yellows disease in canola crops

2.1 Overwintering of infected and non-infected leafhopper populations

As shown in Part 1, *M. quadrilineatus* is the major aster yellows phytoplasma vector in the prairies and *C. humilis* is a phytoplasma carrier and potentially the second most important vector species. While *C. humilis* is known to overwinter in the leaf litter in Saskatchewan (Beirne 1957), *M. quadrilineatus* is not known to overwinter. Knowledge of the presence of overwintering populations of insect vectors and the proportion of infected insects in the overwintering population is important to examine aster yellow disease epidemiology, since infected overwintering insects would emerge early in the spring and spread the disease prior to the arrival of migrant insects from the USA. One of the objectives of the project was to determine if *M. quadrilineatus* and other species known to be phytoplasma vectors could overwinter in Saskatchewan.

2.1.1 Materials and methods

2.1.1.1 Emergence traps

In 2001-2003, emergence traps were placed in fields Brooksby and Medstead and at a site at the AAFC-Saskatoon Experimental Farm. Emergence traps were set up from the beginning of April until the end of May or the end of June depending on the weather.

Emergence traps consisted of 5 L lidded bottomless plastic containers with two side holes covered with a fine mesh to allow air circulation (Figure 4). The containers were slightly pressed into the soil. A sticky card was placed inside each container along the side of the container to catch emerging insects (Figure 4). Emergence traps were placed mostly at the edge of the fields. The sticky traps were changed weekly and the insects were counted and identified.

In 2001, 15-20 emergence traps were placed per field in five canola fields near Medstead . These fields had shown a high incidence of aster yellows in 2000.

In 2002, emergence traps were set up at three locations (pasture, bushes, edges of fields) at the AAFC-Saskatoon Experimental Farm (10 traps per location for a total of 30 traps) and in two fields of caraway (*Carum carvi* L.) near Brooksby (10 traps at the edge of the fields and 10 traps inside the fields for a total of 40 traps). No aster yellows disease had been reported at the Saskatoon site, while the caraway fields near Brooksby had shown 80% aster yellows incidence in 2001 (C.Y. Olivier, unpublished).

In 2003, emergence traps were set up at the same locations than in 2002 at the Saskatoon site (10 traps per location for a total of 30 traps) and in a cereal field and a dandelion glade near Medstead with no aster yellows occurrence in 2001 and 2002. For the cereal field, there were 10 traps on three sides of the field and 10 traps inside the field, placed 100 paces apart, for a total of 40 traps. Twenty traps were set up in the dandelion glade, about 20 metres apart.

2.1.1.2 Pitfalls

Pitfalls consisted of 1 L plastic containers containing a commercial antifreeze solution. Containers were buried into the soil with the opening flush with the soil surface (Figure 5). A plastic funnel was inserted inside each container to prevent the insects that had fallen into the trap from climbing out. The antifreeze solution was filtered each week to retrieve the insects.

In September 2001, 15 pitfalls were set up at the Saskatoon site (pasture, bushes, field edges) and 20 pitfalls were set up in a caraway field near Brooksby (10 traps in the field and 10 traps at the edges of the field) to detect leafhopper activity during late fall and early spring. Insects trapped in the pitfalls were removed once in October and once in November, and weekly from April 15 to June 15, 2002.

2.1.1.3 Suction trap

In 2001 and 2002, a suction trap was set up near Medstead to monitor migrating leafhoppers. The suction trap consisted of an 8-foot tall metal cylinder (30 inch diameter) placed on a wooden tripod to hold it in place (Figure 6). The cylinder was equipped with a suction device that trapped insects which

were then collected in a jar filled with a commercial solution of antifreeze. The antifreeze solution was filtered each week to retrieve the insects.

2.1.1.4 Sweeping nets

In 2001-2003, sweeping nets were used (Figure 7) in every field where emergence traps and pitfalls had been set up to determine if the presence of insects in the fields early in the spring was the result of emergence from overwintering. Twenty 180° sweeps were performed around each emergence trap and pitfall. The collected insects were placed in plastic bags and stored at -20°C until identification could be performed.

All insects collected in the emergence traps and with sweeping nets were identified and analysed individually for the presence of phytoplasma DNA with PCR technology. The insects trapped in the pitfalls and the suction trap were identified. PCR analysis could not performed on these insects since the antifreeze solution damaged the insect tissues and DNA.

2.1.2 Results

Leafhopper species caught in the emergence traps at the sampling sites near Medstead, Brooksby and at the Saskatoon site in the course of the project are listed in Table 4.

In 2001, seven adults and one nymph of *C. humilis* were caught in the emergence traps near Medstead from April 27 to May 30. The first adult of *C. humilis* trapped with a sweeping net near Medstead was caught on May 2. No overwintering adult of *M. quadrilineatus* was found. The first *M. quadrilineatus* adult was trapped near Medstead with a sweeping net on May 30. Adults of *M. quadrilineatus* were first found in the suction trap on May 23.

In 2002, three adults and two nymphs of *C. humilis* were caught in the emergence traps near Brooksby from April 26 to May 14. Two adults of *M. quadrilineatus* were caught in emergence traps at the Saskatoon site from May 20 to May 27. Adults of *M. quadrilineatus* were caught with sweeping nets near Brooksby starting May 23 and at the Saskatoon site starting June 5. The first adult of *M. quadrilineatus* was caught in the suction trap on May 31.

Few insects were trapped in the pitfalls placed near Brooksby and at the Saskatoon site from October 2001 to mid-April 2002, suggesting that there was little insect activity from late fall to early spring. However, more than 1,000 adults of *C. humilis* were trapped in the pitfalls from April 15 to June 6 at both sampling sites while only 11 adults of *M. quadrilineatus* were trapped during the same period.

In 2003, five overwintering adults of *C. humilis* were trapped from April 29 to May 30 near Medstead and four were trapped at the Saskatoon site. The first adult of *C. humilis* was caught with a sweeping net during the last week of April. Four overwintering adults of *M. quadrilineatus* were trapped at the Saskatoon site during the week of May 26 to June 4, while none were trapped near Medstead. The first adults of *M. quadrilineatus* were caught with sweeping nets on May 26 at the Saskatoon site and on June 5 near Medstead.

PCR tests were performed on the six overwintering adults of *M. quadrilineatus*, the 19 overwintering adults and the three nymphs of *C. humilis* and 11 other overwintering hemiptera (Table 4). Of all of the samples, only one overwintering adult of *C. humilis* tested positive for phytoplasma DNA.

2.1.3 Discussion

For *M. quadrilineatus*, approximately 130 degree-days above 9°C are required for egg maturation based on temperature at grass level, and an additional 270 degree-days are required for development to adulthood (Chaput 2004). Therefore, 400 degree-days above 9°C are required for the complete development of a leafhopper adult from the egg stage.

In 2001, near Medstead, western and southeastern winds were blowing from May 20 to May 31 at high altitudes, making it impossible for migrant leafhoppers from the southern USA to land. The fact that no leafhopper adults or nymphs were trapped before May 23 and that the 400 degree-days required to reach adulthood was not reached until June 28 is a strong indication that the *M. quadrilineatus* adults captured near Medstead between May 23 and May 30 originated from overwintering populations.

Adults of *M. quadrilineatus* were found in emergence traps near Brooksby in 2002 and at the Saskatoon site in 2002 and 2003, demonstrating that the aster leafhopper can overwinter as adults in Saskatchewan. As only a small area of soil was covered by the emergence traps and at least two adults were trapped each year, this suggests that the population of overwintering adults could be significant.

The emergence period of adult insects caught with sweeping nets coincided in 2002 and 2003. The 400 degree-days required for the development of a leafhopper adult from the egg stage was not reached until July 8 in 2002 and June 27 in 2003, indicating that the leafhoppers caught with sweeping nets at the end of May did not come from overwintering eggs. In 2002, strong winds originating in Louisiana and Texas on May 19 and 20 were blowing at low altitudes in Saskatchewan from May 21 to May 23, at the same time as the emergence of overwintering leafhoppers, preventing us from differentiating migrant from local leafhopper populations. In 2003, strong southern winds from Texas towards Wisconsin, Ohio and Illinois occurred at the end of May, probably bringing migrant leafhoppers in these states. Southeastern winds blowing over these states at the beginning of June may have brought leafhoppers to Saskatchewan on June 6 and 7. As these winds arriving in Saskatchewan were not directly from the southern USA, the number of migrant leafhoppers was probably low. However, no southern wind was recorded near Brooksby or the Saskatoon site until mid-June, indicating that most of the leafhoppers trapped with the sweeping nets at the end of May originated from local populations.

Adults of *C. humilis* were trapped in high numbers in emergence traps and pitfalls placed near Brooksby in 2002, suggesting that the spring population of *C. humilis* is mainly represented by overwintering adults. In contrast to *C. humilis*, low numbers of *M. quadrilineatus* were found in the traps. This is likely because the adults of *M. quadrilineatus* are mainly flyers while adults of *C. humilis* tend to hop in the leaf litter and hence are more likely to become trapped on the sticky cards or to fall in the pitfalls.

One overwintering adult of *C. humilis* tested positive for phytoplasma DNA, suggesting that the phytoplasma survives the winter in insects in addition to surviving in the roots of perennial plant

species. No overwintering adults of *M. quadrilineatus* tested positive for phytoplasma DNA; the sample size was likely too small to detect infected overwintering adults.

Non-infected overwintering insects can become infected early in the spring by feeding on the phytoplasma reservoirs such as perennial weeds.

The study confirmed that *M. quadrilineatus* can overwinter in Saskatchewan at the adult stage and that there can be high numbers of overwintering insects. This indicates that overwintering adults of *M. quadrilineatus* may be part of the aster yellows reservoir in Saskatchewan, along with infected perennials plants. As long as migrant populations cannot be differentiated from overwintering populations, it will be difficult to determine the exact proportion of overwintering leafhoppers among the global population. Assuming that genetic differences exist between migrant and local populations, differentiating the populations could be achieved with extensive genetic fingerprinting of individual insects.

It is known that adults and nymphs of *C. humilis* can overwinter in high numbers in leaf litter across Saskatchewan. Since this species is a phytoplasma carrier and, considering the high insect density, early emergence in the spring and capability to overwinter as infected adults, the species could become an important vector.

Emergence of *M. quadrilineatus* and *C. humilis* in the spring and frequency of infected insects should be examined as part of monitoring leafhopper populations.

2.2 Leafhopper population survey during the growing season

2.2.1 Materials and Methods

Leafhopper populations were monitored in 2001, 2002 and 2003 from the end of April to the beginning of September at various locations across Saskatchewan (Table 1). Leafhopper monitoring was done weekly or twice a month, depending of the aster yellows history of the area. Sweeping nets, sticky traps and a suction trap were used to sample the leafhopper populations. Sticky traps consisted of sticky cards affixed at 30 cm, 1 m and 1.5 m from the ground on a wooden stake.

Sampling with sweeping nets was performed in 2001, 2002 and 2003 and consisted of taking 20 sweeps at five locations along a transect within each field: 0 m (grass at the edge of the field), 5 m, 10 m, 20 m and 50 m into the field away from any edge. Insects were placed in a plastic bag, kept in a cooler and stored at -18°C.

In 2001, 34 canola fields, seven cereal fields and one caraway field were monitored with sweeping nets. In 2002, 20 canola fields, 16 cereal fields, two caraway fields and five echinacea (*Echinacea* sp.) fields were surveyed. In 2003, 22 canola fields, 18 cereal fields, three echinacea fields and three alfalfa fields were monitored.

To study the distribution of the leafhopper populations in the fields, 30 sticky traps were set up in each of two canola fields near Medstead and in one caraway field near Brooksby in 2001. Ten stakes were placed 100 m apart inside the field. The sticky traps were changed weekly during the growing season (mid-April to mid-September).

The suction trap set up near Medstead in 2001 and 2002 to monitor the leafhopper migration was also used as part of the population monitoring.

2.2.2 Results

Leafhopper species and the number of insects trapped with sweeping nets each year in canola crops are presented in Tables 5, 6 and 7. Over the growing season, among the leafhoppers species that are known to be phytoplasma vectors, *M. quadrilineatus* was the most frequent in canola fields, followed by *E. inimica* (Table 8). Three other species were well-represented in number, namely *C. humilis*, *Psammotettix* sp. and *D. configuratus*. The other species were found in very low numbers. The relative numbers of *M. quadrilineatus* and *C. humilis* insects were similar in 2001 and 2002, but increased in 2003 (Table 8). For the other leafhopper species, the total number of insects decreased from 2001 to 2003. Leafhopper populations near Meadow Lake, Brooksby and Medstead were consistently high from year to year. For the other species, the frequency varied from year to year.

Leafhopper species and the number of insects trapped each year in cereal crops are presented in Tables 10, 11 and 12. *Macrosteles quadrilineatus* was the most frequent leafhopper vector in the cereal fields, followed by *Psammotettix* sp. and *E. inimica* (Table 13). A total of 373 specimens of *Balclutha* sp. were found in cereal fields from 2001 to 2003. *Balclutha* sp. is known to be a carrier of stolbur in Europe, but was not found to be positive for phytoplasma DNA in the present study. Other leafhopper species, including *Athysanus argentarius*, *Doratura stylata*, *Latalus personnatus* and *Verdanus evansi*, were found in high numbers in cereal fields. These species are not known to be phytoplasma carriers or vectors. Nor surprisingly, the specimens were not found to be positive for phytoplasma DNA in the study.

Leafhopper species and the number of insects trapped each year in herb and spice crops are presented in Table 14. *Macrosteles quadrilineatus* and *C. humilis* were the most frequent leafhopper vectors/carriers in echinacea fields, followed by *Psammotettix* sp. and *E. inimica* (Table 15). A total number of 60 specimens of *Balclutha* sp. were found in the echinacea fields from 2001 to 2003. Other leafhopper species, including *A. argentarius*, *V. evansi* and *L. personatus*, were found in high numbers in caraway fields (Table 15).

The evolution of the population of *M. quadrilineatus* in canola and cereal crops over time is shown in Figures 8 and 9. For all three years, the number of adults of *M. quadrilineatus* increased during the growing season. In 2001, in canola, the population reached a peak at the end of June, the end of July and the end of August. In 2002 and 2003, the number of adults reached a peak at the end of June and July. In 2002, there was a small peak at the end of August, while in 2003, there were few insects in canola crops at the end of August, likely because of the unfavourable climatic conditions.

In 2001, cereal crops were surveyed from mid-July to mid-September. The number of adult insects reached a peak at the end of July and again at the end of August. In 2002, the numbers of adult insects were high from the end of June to mid-July and reached another peak at the end of July. Similarly, in 2003, the number of insects reached a peak at the end of June and at the end of July. For both years, there were few insects caught in August.

Large numbers of *M. quadrilineatus* nymphs were found in cereal crops, while few nymphs were observed in canola crops (Table 16).

Larger numbers of *M. quadrilineatus* adults were found inside cereal fields than at the edge of the fields. In contrast, larger numbers *M. quadrilineatus* adults were found at the edge of the canola fields than inside the fields (Table 17).

Sixty-five to 85% of the *M. quadrilineatus* adults were collected in the sticky traps placed at 30 cm from the ground in the canola fields, 12-14% of the adults were trapped on the sticky traps located at

1 m from the ground, and only a few adults were caught at 1.5 m. No nymphs were trapped on the sticky traps in the canola fields. In the caraway field, the percentage of *M. quadrilineatus* caught on the sticky traps at 30 cm, 1 m and 1.5 m were 48%, 34% and 17%, respectively.

Few migrant specimens of *M. quadrilineatus* were caught in the suction trap in 2001 and 2002, providing little information on migrant populations during the growing season.

2.2.3 Discussion

Four leafhopper species known to be phytoplasma carriers or vectors, namely, *M. quadrilineatus*, *S. ulheri*, *E. inimica* and *Psammotettix* sp., were found in high numbers in canola and cereal fields. *Macrosteles quadrilineatus* is the most frequent leafhopper species in both crops. The number of insects of *M. quadrilineatus* was consistently higher in cereal fields than in canola fields, suggesting a feeding preference for cereals. Also, nymphs of *M. quadrilineatus* were mostly found in cereal crops (Table 14), confirming the preference for *M. quadrilineatus* to feed and lay eggs in cereal crops.

Adults and nymphs of *M. quadrilineatus* were found mostly inside the cereal fields and at the edge of canola fields, supporting the preference of the leafhopper for cereals. Field observations over 2002-2003 suggested that leafhoppers have a feeding preference for barley *(Hordeum vulgare* L.), followed by wheat (*Triticum* species) and oats (*Avena sativa* L.). These observations need to be repeated to confirm that barley is more attractive to *M. quadrilineatus* than wheat and oats.

Generally it appears that the aster leafhopper is not attracted by canola crops. When large numbers of *M. quadrilineatus* are found in canola fields, this is likely the result of 1) a high egg population in the field where the canola crop was seeded, particularly in a no-till situation which prevents the eggs from becoming buried into the soil and being subjected to microbial degradation, and 2) high numbers of migrating insects from the USA, as well as favourable climatic conditions.

In 2001-2003, the adult *M. quadrilineatus* insects caught at the end of June (June peak) represented insects having emerged from overwintering eggs, corresponding to the 400 degree-days required for leafhoppers to reach adulthood. The adult insects at the end of July (July peak) originated from eggs laid in the spring by overwintering and migrant adults. The adult insects caught at the end of August in 2001 originated from eggs laid during the summer months. In 2002-2003, there were very few adult insects at the end of August, likely because of the unfavourable dry conditions.

The results indicated that there were two, and possibly three, generations of the aster leafhopper per growing season in Saskatchewan, depending on the climatic conditions.

The results of the study led to the establishment of the cycle of the aster leafhopper, which was published in Diseases of Field Crops in Canada (Bailey et al. 2003).

2.3 Aster yellows disease survey

Symptoms of aster yellows diseases were first described by Kunkel (Kunkel 1926), on American aster plants showing virescence and malformations. Most symptoms consisted of tissue proliferation, stunting, yellowing and virescence (excessive greening of floral tissue) or phyllody (leaf-like sepals and petals) (Seemüller et al. 1998). Aster yellows symptoms were described on oilseed rape

(rapeseed) in western Canada more than 50 years ago (Sackston 1953). Typical symptoms of aster yellows infection in canola include plant stunting, virescence, leaf yellowing, phyllody and bladder-like siliques (Bailey et al. 2003).

2.3.1 Materials and Methods

In 2001-2003, for each sweeping site (Table 1) and visit, 100 canola, 100 caraway and 100 echinacea plants were observed for the presence of aster yellows symptoms. In 2003, 100 plants were also observed in all the cereal fields visited at each sweeping site. Overall, more than 250,000 plants were observed. Plants of other species, e.g., weedy species, shrubs and trees, were also observed and a number of samples were collected.

In 2001-2003, flowering symptomatic and non-symptomatic plants were collected in canola and herb and spice crops, mainly near Kelvington, Brooksby and Medstead. Root, leaf, stem and floral tissue of each plant was freeze-dried and seeds were stored at 4°C. In 2003, non-symptomatic canola seedlings (N=100) and plants at the budding stage (N=25), and cereal plants at all stages of development (N=150) were also sampled.

All canola samples (more than 600) were analysed for the presence of phytoplasma DNA by PCR technology as described in Section 1.1. A modification was made to the protocol in that DNA was extracted from about 1 g fresh weight of tissue or for seeds, from bulks of 5-10 seeds. We examined 250 samples of echinacea and caraway and about 100 additional samples from weeds, shrubs and trees with aster yellows-like symptoms.

2.3.2 Results

Typical aster yellows symptoms were observed on canola (Figures 10 and 11), *Brassica tournefortii*, caraway (Figure 12), coriander (*Coriandrum sativum* L.), echinacea (Figure 13) as well as on weeds such as stinkweed (*Thlaspi arvense* L.), false flax (*Camelina sativa* (L.) Crantz), hawk's beard (*Crepis runcinata* (James) T. & G.) (Figure 14), dandelion (*Taraxacum* sp.) (Figure 15), raspberry (*Rubus* sp.) (Figure 16), sea-buckthorn (*Hippophaë rhamnoides* L.) (Figure 17), saskatoon berry (*Amelanchier alnifolia* Nutt.) and honeysuckle (*Lonicera villosa* (Mitchx.) R. & S.) (Figure 18). Grain crops can also be affected, for instance, barley (Figure 19).

In 2001-2003, the percentage of symptomatic plants did not exceed 1% in canola and 5% in echinacea fields. In 2002, 80% of the plants in a caraway field near Brooksby presented aster yellows symptoms. There were no aster yellows symptoms in cereals, although we detected barley plants infected with phytoplasma DNA among the plants collected in 2003.

No canola seedlings tested positive for phytoplasma DNA and only two plants at the budding stage were found to contain phytoplasma DNA. Fifty of the 150 plants collected in 2003 have been examined to date. Four plants have tested positive for phytoplasma DNA.

Symptomatic plants of dandelion, raspberry, hawk's beard, false-flax and saskatoon berry were observed occasionally. All samples collected on the symptomatic plants tested positive for phytoplasma DNA.

Atypical aster yellows symptoms were also observed in canola crops. The atypical symptoms consisted of stems that were totally or partially purple and which bore normal siliques containing well-formed and small, misshapen seeds (Figures 20 and 21). While siliques of plants with typical aster yellows symptoms contained 40-50% well-formed seeds, siliques of plants with atypical aster yellows symptoms contained 80-100% well-formed seeds.

In 2002, ten plants with atypical symptoms were harvested near Kelvington and PCR analysis was performed on leaf tissue. The analysis revealed that eight of the 10 plants were infected with phytoplasmas. In 2003, ten plants with atypical aster yellows symptoms were harvested near Medstead; four of the plants tested positive for phytoplasma DNA.

The results obtained with the seed collected on canola plants with typical and atypical symptoms are presented in Part 4.

2.3.3 Discussion

The presence of infected, non-symptomatic canola and cereal plants is due to the latency period required by a phytoplasma-infected plant to express symptoms. Depending of the plant species, the latency period can last 5-6 weeks for canola up to a few years for perennial plants, shrubs and trees. The aster yellows phytoplasma seems to induce a series of disorders, including phloem cell hyperplasia, alteration of sap transport, and hormonal disturbances (Chang 1998). Symptoms such as phloem hyperplasia occurs only when large numbers of phytoplasmas are present in the phloem (Oshima et al. 2001).

Canola plants with atypical aster yellows symptoms were found to be infected by phytoplasmas. The atypical symptoms consisted of a purplish main stem bearing partially filled siliques. The observation of fewer well-formed seeds in plants showing typical aster yellows symptoms than in plants exhibiting atypical symptoms probably relates to the time of infection. Plants that become infected early in the season are more likely to develop the typical aster yellows symptoms commonly observed at maturity and to have reduced seed set. These atypical symptoms should be added to the list of symptoms that are recorded in field surveys for aster yellows disease.

From 2001-2003, several biannual or perennial plants species such as hawk's beard and dandelion were commonly found in or near crops and were found to be infected with phytoplasmas. These weeds, as well as many other species of plants that have been identified as hosts of the aster yellows phytoplasma, can act as reservoirs for the disease. The presence of wild alternate hosts in or near canola fields influences the density of leafhoppers and the incidence of the disease in the crops by providing feeding and breeding sites for the leafhoppers during periods of dispersal or when preferred crops are unavailable. This means that canola plants grown in a weedy field have a greater chance of becoming infected by phytoplasmas than canola plants located in a weed-free field.

Barley, wheat, oats and rye, as well as biannual herb and spice plants such as caraway, echinacea and coriander, are alternate hosts for the aster yellows phytoplasma. Herb and spice plants are known to be a source of disease for surrounding crops and to act as phytoplasma reservoirs. Also, our observation of non-symptomatic infected barley plants in barley fields suggests that barley crops can be a source of phytoplasma inoculum for surrounding canola fields.

The presence of very high populations of *M. quadrilineatus* in cereal and herb and spice crops increases the chance of disease in subsequent canola crops, especially in a no-till situation, which favours overwintering of leafhopper eggs.

From 2001-2003, the incidence of aster yellows disease in canola crops was very low. In 2001, the percentage of plants showing aster yellows symptoms was less than 1%, in spite of the 2000 epidemic which probably resulted in high numbers of infected perennial plants and high numbers of infected overwintering leafhopper adults. In 2001, a high proportion of infected adults of *M. quadrilineatus* was observed in most areas (Table 18), especially in early spring (data not shown). The low disease incidence was probably caused by the high number of days with temperatures above 30°C. High temperatures have a direct effect on phytoplasma survival and the ability of infected leafhoppers to transmit the phytoplasma. Kunkel (1937) reported that if infective leafhoppers were exposed to temperatures between 31°C and 38°C for 12 days or longer, their ability to transmit the phytoplasma was lost. Also, Caudwell (1966) reported that 80% of infected plants placed at 30°C for 72 hours were cured. In 2001, temperatures above 30°C were recorded for 10 days in July, 13 days in August (with 5 consecutive days with temperatures maxima reaching 35°C) and 4 days in September.

Again, in 2002, low disease incidence was recorded despite the high percentage of infected leafhoppers. This is likely due to the dry, hot climatic conditions which did not favour transmission of the phytoplasma. In 2003, low percentage of infected leafhoppers and the absence of strong winds directly originating from the southern USA are likely the causes of the very low disease incidence observed.

The research performed during the course of the grant has allowed the description of the aster yellows disease cycle, including the life cycle of the major vector, *M. quadrilineatus* (Figure 22).

PART 3 – Aster yellows germplasm screening

3.1 Leafhopper rearing

Adults of *M. quadrilineatus* captured in the field in 2001 were placed in cages. Successive generations of healthy leafhoppers have been obtained since 2001. Leafhoppers reproduce successfully on leaves of barley, carrot (*Daucus carota* L.), and lettuce (*Lactuca sativa* L.) completing their life cycle in 21 to 28 days. The leafhoppers fed on false flax, but there is no evidence of oviposition on this crucifer.

Attempts to develop a colony of phytoplasma-infected insects were repeatedly made. Healthy leafhoppers were placed on field-grown infected plants of echinacea and carrots, but none of the insects became infected with the phytoplasma. We also placed field-caught leafhoppers on healthy barley plants and screened for the development of aster yellows symptoms. None of the plants developed disease symptoms, indicating that the insects, which were confirmed post-mortem as being infected wih phytoplasmas) were non-infectious.

3.2 Germplasm screening

Screening canola germplasm for resistance to aster yellows could not be undertaken because we were unable to establish a colony of infected *M. quadrilineatus*. This is likely because the trapped leafhoppers were either not infected or not infectious at the time of collection in August. It has been reported for the grapevine leafhopper that infectivity follows a seasonal pattern and that leafhoppers are more infectious in early spring and lose their infectivity later on (Tanne et al. 2001). It remains to be seen if such a seasonal pattern exists in Saskatchewan. Also, the dry, hot conditions experienced may have rendered the leafhoppers non-infectious, although they remained infected as the data collected in the present study indicates.

PART 4 – Transmission of phytoplasmas

4.1 *Brassica napus*

In 2002, ten plants with typical aster yellows symptoms, ten plants with atypical aster yellows symptoms and two symptomless plants were collected at maturity in a canola field near Kelvington. Phytoplasma DNA was detected in the leaf, root and stem tissue of the ten plants with typical aster yellows symptoms and in eight of the ten plants with atypical aster yellows symptoms. The two symptomless plants tested negative.

Ten of 50 samples of well-formed seeds and 35 of 153 samples of misshapen seeds from the plants with typical symptoms tested positive for phytoplasma DNA, while only one of 150 samples of well-formed seeds and none of the 72 samples of misshapen seeds from the plants with atypical symptoms tested positive for phytoplasma DNA.

RFLP analysis revealed that the phytoplasma-positive seeds were infected only with phytoplasmas belonging to aster yellows strain 16SrI-B. To our knowledge, this is the first time that phytoplasmas have been detected in canola seeds.

4.2 *Brassica rapa*

In 2003, four plants with typical aster yellows symptoms and one plant with atypical symptoms were collected in a *B. rapa* canola field near Medstead. Leaf tissue of the five plants tested positive for phytoplasma DNA.

Plant-1 had 10% misshapen seeds and a 96% germination rate for the well-formed seeds. Plant-2 had 80% misshapen seeds and a 53% germination rate for the well-formed seeds. Plant-3 and Plant-4 had 30% misshapen seeds and a 90% germination rate for the well-formed seeds. Plant-5 (atypical symptoms) had 10% misshapen seeds and a 67% germination rate for the well-formed seeds. None of the misshapen seeds germinated.

The presence of phytoplasma DNA was examined in ten samples of five well-formed seeds and ten samples of five misshapen seeds for each of the five plants. All samples tested negative for phytoplasma DNA, with the exception of the misshapen seed samples of Plant-1. This suggests that, in this plant, the phytoplasmas were translocated to developing seed from the infected leaf tissue, since leafhoppers are not known to feed on flowers.

To investigate whether phytoplasmas in seed could be further transmitted to progeny plants, additional seeds from the five plants were germinated and the progeny plants (N=40 per progeny for a total of 200 plants) were raised in the greenhouse alongside plants from the original seedlot. Cotyledon tissue was sampled as well as leaf tissue on plants at the early flowering and late maturity stage on all plants. All samples were tested for the presence of phytoplasma DNA using PCR technology.

In the course of this work, it was observed that in contrast to the plants grown from the original seedlot, there were abnormal-looking plants in the progeny of the three of the five original phytoplasma-infected plants, including plants that had no growing point (Figure 23), plants that had stocky and twisted stems (Figure 24), plants that had large and hairy leaves with a high number of trichomes (Figure 25) and plants that had compressed inflorescences (Figure 26).

The growth of all of the progeny plants was delayed by a minimum of 3 weeks (Figure 27) relative to that of the plants from the original seedlot. Progeny plants of Plant-3 and Plant-5 were all normal looking, but their growth was delayed. Among progeny of Plant-1, five plants had no growing point and 12 plants had stocky and twisted stems with very large and hairy leaves carrying a high number of trichomes. Among progeny of Plant-2, two plants had no growing point and five plants had twisted stems and very large leaves. Among progeny of Plant-4, only two plants had no growing point. All the plants that had no growing point eventually developed a very stocky stem bearing inflorescences that were very compressed.

Recently, Starzycki et al. (2003) reported the occurrence of malformed plants in the self-progeny of phytoplasma-infected plants of winter-type *B. napus*. The authors could not detect the presence of phytoplasmas in the malformed plants and postulated that the phytoplasmas in the parental plants had induced genetic changes that had been passed to the progeny. Although this result remains to be confirmed, it parallels our observations in *B. rapa*. Indeed, PCR analysis revealed that none of the abnormal-looking plants were positive from phytoplasma DNA. Only two normal-looking progeny plants from Plant-1 and three normal-looking progeny plants from Plant-2 were positive for phytoplasma DNA. Research is continuing to determine phytoplasmas in plant tissue can induce genetic changes in developing seeds and whether the phytoplasmas in infected seeds can be translocated to the developing plant.

The results of the various studies have generated epidemiological information required for the development of technologies for early-warning systems and pest management strategies. We can anticipate the infestation of canola crops based on the leafhopper frequency in the previous crop, the wind pattern from mid-May to mid-June, and the climatic conditions. The low level of aster yellows in canola during the project did not enable us to establish an economical threshold for leafhopper infestations and the frequency of infective insects.

The research has also led to new avenues of investigation, including seed transmission of the aster yellows disease and the potential genetic effects of the aster yellows phytoplasma on plant growth and development.

Literature cited

Bailey, K.L., Gossen, B.D., Gugel, R.K., and Morrall, R.A.A. (Eds.). 2003. Diseases of Field Crops in Canada, 3rd Edition. The Canadian Phytopathological Society, Saskatoon, Saskatchewan, Canada.

Beanland, L. and Wolf, T. 2003. Possible insect vectors of North America grapevine Yellows phytoplasma in Virginia. Proceedings of the 14th ICVG Conference, 12-17 September 2003, Locorotondo, Italy. pp. 64-65.

Beirne, B.P. 1956. Leafhoppers (Homoptera: Cicadelliade) of Canada and Alaska. Can. Entomol. 88 (Suppl. 2):180.

Benard, D., Lange, R.M., and Harrison, L.M. 2001. Survey of fusarium wilt and other canola diseases in Alberta, 2000. Can. Plant Dis. Surv. 78:102-103.

Caudwell, A.1966. L'inhibition in vivo des virus de la Flavescence dorée par la chaleur. Annales des épiphyties, Hors série n°17, Etudes de virologie, pp. 61-66.

Chang, C.-J. 1998. Pathogenicity of aster yellows phytoplasma and *Spiroplasma citri* on periwinkle. Phytopathology 88:1347-1350.

Chaput, J. 2004. The aster leafhopper and aster yellows. Adgex # 251/605. www.gov.on.ca/omafra/english/crops.facts.98-057.htm

Chiykowski, L.N. 1965. A yellow type virus of alsike clover in Alberta. Can. J. Bot. 43:527-404.

Chiykowski, L.N. 1981. Epidemiology of diseases caused by leafhopper-borne pathogens. In: Plant Disease and vVctors, K. Maramorosch and K.F. Harris (Eds.), Academic Press, New York, USA. pp. 106-159.

Chiykowski, L.N. and Chapman, R.K. 1965. Migration of six-spotted leafhopper *Macrosteles fascifrons* (Stål). Part 2. Migration of six-spotted leafhopper in central North America. Univ. Wisconsin-Madison Coll. Agric. Life Sci. Res. Bull. 261:21-45.

Chiykowski, L.N. and Sinha, R.C. 1990. Differentiation of MLO diseases by means of symptomatology and vector transmission. Zentrabl. Bakteriol. Suppl. 20:280-287.

Daire, X., Clair, D., Reinert, W. and Boudon-Padieu, E. 1997. Detection and differentiation of grapevine yellows phytoplasma belonging to the elm yellows group and to the stolbur subgroup by PCR amplification of non-ribosomal DNA. Eur. J. Plant Pathol. 103:507-514.

Davis, D.L. and Eyre, S. 1996. Detection of phytoplasmas associated with pear decline in pear psyllids by polymerase chain reaction. In: Brighton Crop Protection Conference Symposium Proc. No 65: Diagnostic in Crop Protection, F.M. McKim (Ed.), British Crop Protection Council, Surrey, UK. pp. 67-72.

Griffith, H.M., Sinclair, W.A., Boudon-Padieu, E., Daire, X., Lee., I.M., Sfalanga, A. and Bertaccini, A. 1999. Phytoplasmas associated with elm yellows: Molecular variability and differentiation from related organisms. Plant Dis. 83:1101-1104.

Hill, G.T. and Sinclair, W.A. 2000. Taxa of leafhoppers carrying phytoplasmas at sites of Ash Yellows occurrence in New York state. Plant Dis. 84:134-138.

Hiruki, C. 1998. Recent advances in Paulownia witches'-broom research. Eur. J. For. Pathol. 28:81.

Hoy, C.W., Heady, S.E. and Koch, T.A. 1992. Species composition, phenology and possible origins of leafhoppers (Cicadellidae) in Ohio vegetable crops. J. Econ. Entomol. 85:2336-2343.

Khadhair, A.-H., Hiruki, C. and Hwang, S.F. 1997. Molecular detection of alfalfa witches'broom phytoplasma in four leafhopper species associated with infected alfalfa plants. Microbiol. Res. 152:269-275.

Khadhair, A.H., McClay, A., Hwang, S.F. and Shah, S. 1999. Aster yellows phytoplasma identified in scentless chamomile by microscopical examinations and molecular characterization. J. Phytopathol. 147:149.

Khadhair, A.H., Tewari, J.P., Howard, R.J. and Choban, B. 2000. Association of aster yellows and witches'-broom phytoplasmas with several plant species in Alberta. Can. J. Plant Pathol. 23:185.

Kunkel, L.O. 1926. Studies on aster yellows. Am. J. Bot. 13:646-705.

Kunkel, L.O. 1937. Effect of heat on ability of *Cicadula sexnotata* (Fall.) to transmit aster yellows. Am. J. Bot. 24:316-327.

Lee, I.-M., Gundersen-Rindal, D.E. and Bertaccini, A. 1998. Phytoplasma L: Ecology and genomic diversity. Phytopathology 88:1359-1366.

Lee, I.-M., Martini, M., Bottner, K.D., Dane, R.A., Black, M.C. and Troxclair, N. 2003. Ecological implications from a molecular analysis of phytoplasmas involved in an aster yellows epidemic in various crops in Texas. Phytopathology 93:1368-1377.

Marzachi, C., Veratti, F. and Bosco, D. 1998. Direct PCR detection of phytoplasmas in experimentally infected insects. Ann. Appl. Biol. 133:45-54.

McCoy, R.E., Caudwell, A., Chang, C.J., Chen, T.A., Chiykovsky, L.N., Cousin, M.T., Dale, J.L., de Leeuw, G.T.N., Golino, D.A., Hacket, K.J., Kirkpatrick, B.C., Marvitz, R., Petzold, H., Sinha, R.C., Sugiura, M., Whitcomb, R.F., Yang, I., Zhu, B.M. and Seemüller, E. 1989. Plant diseases associated with mycoplasma-like-organisms. In: The Mycoplasmas. Volume 5. Spiroplasmas, Acholeplasmas, and Mycoplasmas of Plants and Arthropods, R.F. Whitcomb and J.C. Tully (Eds), Academic Press, New York, USA. pp. 545-640.

Oshima, K., Shiomi, T., Kuboyama, T., Sawayanagi, T., Nishigawa, H., Kakizawa, S., Miyata, S.-I., Ugaki, M. and Namba, S. 2001. Isolation and characterization of derivatives lines of the onion yellows phytoplasma that do not cause stunting or phloem hyperplasia. Phytopathology 91:1024-1029.

Pearse, P.G., Morrall, R.A.A., Kutcher, R., Kaminski, D.A., Duczek, L.J., Gugel, R.K., Rimmer, S.R. and Berard, R. 2000. Survey of canola diseases in Saskatchewan, 1999. Can. Plant Dis. Surv. 77:81-82.

Pearse, P.G., Morrall, R.A.A., Kutcher, R., Keri, M., Kaminski, D.A., Gugel, R.K., Anderson, K., Trail, C. and Greuel, W. 2001. Survey of canola diseases in Saskatchewan, 2000. Can. Plant Dis. Surv. 78: 105-106.

Sackston, W.E. 1953. Rape. Can. Plant Dis. Surv. 33: 41.

Seemüller, E., Marcone, C., Lauer, U., Ragozzino, A. and Göschl, M. 1998. Current status of molecular classification of the phytoplasmas. J. Plant Pathol. 80:3-26.

Starzycki, M., Starzycka, E., and Pszczola, J. 2003. Deformations of rapeseed *Brassica napus* L. plants in the first progeny after phytoplasma infection. In: Proceedings of the 11th International Rapeseed Congress. 6-10 July 2003, Copenhagen, Denmark. The Royal Veterinary and Agricultural University, Frederiksberg, Denmark. pp. 1109-1111.

Tanne, E., Boudon-Padieu, E., Clair, D., Davidovich, M., Melamed, S. and Klein, M. 2001. Detection of phytoplasma by polymerase chain reaction of insect feeding medium and its use in determining vectoring ability. Phytopathology 91:741-746.

Tsai, J.H. 1979. Vector transmission of mycoplasmal agents of plant diseases. In: The mycoplasmas. Volume 3. Plant and Insect Mycoplasmas. R.F. Whitcomb and J.G. Tully (Eds.), Academic Press, New York, USA. pp. 265-307.

Vanterpool, T.C. 1963. Rape diseases in Saskatchewan in 1963. Can. Plant Dis. Surv. 43:212-214.

Wang, K. and Hiruki, C. 2001. Molecular characterization and classification of phytoplasmas associated with canola yellows and a new phytoplasma strain associated with dandelions. Plant Dis. 85:76-79.

Weber, A. and Maixner, M. 1998. Survey of populations of the planthopper *Hyalesthes obsoletus* Sign. (Auchenorrhyncha: Cixiidae) for infection with the phytoplasma causing grapevine yellows in Germany. J. Appl. Entomol. 122:375-381.

Zreik, L., Danet, J.-L., Fos, A., Gandar, J., Bove, J. and M. Garnier. 1990. Detection of tomato stolbur and clover phyllody MLOs in plants and insects with monoclonal antibodies. In Proceedings of the 5th International Congress of the IOM, 8-12 July 1990, Istanbul, Turkey. pp. 225-226.

[1]Dr. C.Y. Olivier was hired to work on the project because of her broad expertise on leafhoppers and the aster yellows disease.

[2]S. Pooler was an AAFC-SRC FSWEP student who assisted Dr. Olivier with the monitoring and collection of leafhoppers and other insects caught in traps and sweeps. Ms. Pooler was subsequently hired as a casual part-time technician to assist Dr. Lefol with the identification of insects collected in 2001.

[3]G. Murza was an AAFC-SRC casual technician who assisted Dr. Olivier with the identification of insects collected in 2002 and 2003.

[4]A. Séguin was an AAFC-SRC FSWEP student who assisted Dr. Olivier with the monitoring and collection of leafhoppers and other insects caught in traps and sweeps.

[5]Others

AAFC-Saskatoon Research Centre Research Technicians who participated in the project:

M. Braun, B. Galka, L. Grenkow, L. Oliver, D. Sargeant, R. Weiss, S. Woods (disease and insect survey)

R. Andrahennadi (insect identification)

Project Developed Materials

Research publications and other published information

1) Olivier-Lefol, C. 2003. Life cycle of aster yellows. In: Diseases of Field Crops in Canada, 3rd Edition, Bailey, K.L., Gossen, B.D., Gugel, R.K. and Morrall, R.A.A. (eds.). The Canadian Phytopathological Society, Saskatoon, Saskatchewan.

2) Olivier-Lefol, C.Y. 2004. Aster Yellows. In: Compendium of *Brassica* Diseases, Rimmer, R.S., Shattuck, V. and Buchwaldt, L. (Eds.). The American Phytopathological Society Press (in press)

3) Topinka, K., Olivier-Lefol, C.Y. and Maurice, D. 2004. Comparison of Group 2 residual injury symptoms in canola. Herbicide residue - or is it? Country Guide, March 2004 issue (in press).

4) Olivier, C.Y., Séguin-Swartz, G., Olfert, O.O., Murza, G. and Seidle, E. 2004. Unknown cause of malformations in canola crops in Western Canada in 2002 and 2003. Cruciferae Newslett. No. 26: (in press)

5) Olivier, C., Séguin-Swartz, G., Murza, G., Boudon-Padieu, E. and Olfert, O.O. 2004. Molecular detection of aster yellows in seed of canola (*Brassica napus*). Can. J. Plant Pathol. (Manuscript submitted)

Communications

a) Presentations

1. Lefol, C.Y. 2001. Aster yellows in canola. Skyfarm Office, 22 June 2001, Canora, Saskatchewan.
2. Lefol, C.Y. 2001. Aster yellows in canola. 37th Annual Meeting of the Saskatchewan Advisory Sub-Council on Insect Control, 9 November 2001, Saskatoon, Saskatchewan.
3. Lefol, C.Y. 2001. Presentation on the 2001 aster yellows status in Saskatchewan. Saskatchewan Advisory Council on Crop Protection (SACCP) Plant Disease Sub-Council Meeting, 29 November 2001, Saskatoon, Saskatchewan.
4. Lefol, C.Y. 2001. Pest status of leafhoppers and their impact on aster yellows disease in canola. Canola Industry Meeting, 5 December 2001, Saskatoon, Saskatchewan.
5. Olivier-Lefol, C.Y. 2002. Aster yellows in herb and spice crops. Presentation made at Mr. T. Heibeit's farm, 23 April 2002, Brooksby, Saskatchewan.
6. Olfert, O., Olivier-Lefol,C. and Séguin-Swartz, G. 2002. Relationship between leafhoppers and aster yellows disease in canola. Report to Western Committee on Crop Pests, 4-5 October 2002, Winnipeg, Manitoba.
7. Olivier-Lefol, C.Y. 2002. Aster yellows research update. Canola Industry Meeting, 3 December 2002, Saskatoon, Saskatchewan.

8. Olivier-Lefol, C.Y. 2003. Are FPA symptoms in canola caused by phytoplasma? Presentation made at a meeting of the Saskatchewan Canola Development Commission, 21 October 2003, Saskatoon, Saskatchewan.
9. Olivier-Lefol, C.Y. 2003. Are FPAs caused by phytoplasmas?. Canola Seedling Issues Workshop, 30 October 2003, Kelowna, British Columbia.
10. Olivier-Lefol, C.Y. 2003. Aster Yellows. Canola Industry Meeting, 3 December 2003, Saskatoon, Saskatchewan.

b) Technology Transfer

1) Phone calls (more than 50 calls) from farmers and representatives of the Saskatchewan Department of Agriculture and Food requesting information about aster yellows epidemiology and control. Numerous communications in particular with K. Turkington (AAFC-Lacombe), K. Topinka (Alberta Agriculture and Food Development, Edmonton), M. Hartman (Alberta Agriculture, Food and Rural Revitalization, Regina) and L. Hall (Alberta Agriculture and Food and Rural Development, Edmonton), R. Pidskalny (BASF, Edmonton), P. Pearse (Saskatchewan Agriculture, Food and Rural Revitalization, Regina), T. Meyers (Agricore United, Saskatoon) and D. Maurice (Westco, Calgary).

2) Several presentations (about 20, 2001-2003) to producers on aster yellows in canola and caraway.

3) Provided PowerPoint presentation regarding the canola seedling issues to K. Topinka (Alberta Agriculture, Food and Rural Development) for talks given at the Canola Industry meeting, ARC Edmonton (November 12, 2003), at the Alberta Weed Advisory Committee, Edmonton (November 13, 2003), at the Canola Council of Canada - Canola Production Committee, Winnipeg (November 18, 2003) and at the Agronomy Update, Red Deer (January 13, 2004).

c) Media

1) Interview with Bruce Barker for an article published in the Canola Guide:
Barker, B. 2001. Aster yellows infests late crops. Canola Guide, April 2001 issue, pp. 24-25.
2) Interview by Jack Daws on Dawson Manitoba Radio CKDM about aster yellows disease on canola. Interview recorded and broadcasted on 16 July 2001.
3) Interview by Kelly Funke for an article published in the Canola Digest:
Funke, K. 2003. Scientist seek cause of mysterious canola damage, September / October issue, p. 9.
4) Interview by Jay Whetter for an article in the Canola Guide:
Whetter, J. 2003. Strange growths stymie canola pathologists,. Canola Guide October/ November issue, p. 7.

d) Illustrations

1) 35 mm slides illustrating the aster yellows leafhopper and other leafhoppers, aster yellows disease symptoms, insect trapping methods, etc., were developed.
2) PowerPoint presentation on aster yellows.
3) Life cycle of the aster yellows disease and illustrations of plants exhibiting aster yellows symptoms published in the third edition of "Diseases of Field Crops in Canada", published by the Canadian Phytopathological Society, Saskatoon, Saskatchewan.
4) Illustrations of canola plants exhibiting aster yellows symptoms published in the Compendium of *Brassica* Diseases, published by the American Phytopathological Society Press (in press)

Acknowledgements

Funding provided by Saskatchewan Agriculture Development Fund was acknowledged when discussing the project with public and private sector visiting scientists and in all communications at scientific conferences and workshops and producer meetings.

The collaboration of the following Saskatchewan producers is gratefully acknowledged:

E. Seidle (Medstead)
G. Conrad (Estevan)
G. Detulieu (Vonda)
D. Garnet (Medstead)
R. Gilander (Elrose)
B. Hardyi (Norquay)
T. Heibeit (Brooksby)
D. Kachur (Lanigan)
The Lundberg Family (Turtleford)
D. Ornawka (North Battleford)
J. Russel (Meadow Lake)
K. Sapsford (Biggar)
T. Senko (Lanigan)
Spruce Lanes Acres (Kelvington)
M. Yaholnitsky (Canora)

The authors acknowledge the scientific contribution of Dr. A. Hamilton, AAFC- Eastern Cereal and Oilseed Research Centre, Ottawa, who provided expert confirmation of the insect identification, the assistance of Dr. V. Ripley, AAFC-SRC and his staff in providing laboratory facilities, equipment and expert advice for the PCR analyses, the assistance of Dr. E. Boudon-Padieu, Unité Mixte de Recherche 1088 Plante Microbe Environnement, INRA-Université de Bourgogne, Dijon, France, and her staff whose collaboration allowed us to perform the RFLP research. We also acknowledge the expert assistance of photography and computer graphics specialist R.E. Underwood, AAFC-SRC in preparing slides and illustrations.

Table 1. Location of the sites surveyed and aster yellows (AY) history. (Crops indicated in italics were not surveyed.)

Region / Field location	Aster yellows in canola, caraway and echinacea crops in 2000	Year of aster yellows survey		
		2001	2002	2003
Locations near Medstead				
Medstead (RM 497)				
NW30-50-14	10% AY - Canola	*Cereal*	Barley	Canola
NE15-51-14	25% AY - Canola	*Barley*		
NW14-51-14	25% AY - Canola	Barley / Canola		
NW32-50-14	10% AY - Canola	*Cereal*		
NW22-50-14	40% AY - Canola	*Cereal*		
SW31-50-14	No aster yellows observed	*Barley*	Canola	Barley
NW20-50-14	No aster yellows observed	*Barley*	Canola	Barley
NE7-50-14	No aster yellows observed	*Canola*	Oats	Oats
SE25-50-14			*Summer Fallow*	Canola
Parkdale (RM 498)				
SE13-45-16		*Summer Fallow*	Canola	

Table 1 (Continued)

Region Field location	Aster yellows in canola, caraway and echinacea crops in 2000	Year of aster yellows survey		
		2001	2002	2003
Locations near North Battleford Battle River (RM 438)				
NE9-44-18	No aster yellows observed	Canola		
NW33-43-16	No aster yellows observed	Canola		
NE13-45-17			Canola	
NE23-45-17			Canola	
NW24-45-18	No aster yellows observed	*Canola*	Wheat	
Locations near Turtleford Turtleford (RM 499)				
SW30-50-21	No aster yellows observed	Barley	Canola	Barley
NW 30-50-21	No aster yellows observed		Barley	Canola
Frenchman Butte (RM 501)				
SE34-50-22	No aster yellows observed	Barley	Canola	Barley
Locations near Meadow Lake Meadow Lake (RM 588)				
SE10-60-14	30% AY - Canola	wheat	wheat	Canola
NW12-60-14	30% AY - Canola	wheat	barley	Canola

Table 1 (Continued)

Region / Field location	Aster yellows in canola, caraway and echinacea crops in 2000	Year of aster yellows survey		
		2001	2002	2003
SW12-60-14		Wheat	Canola	Wheat
SE11-60-14		Canola	Wheat	Barley
NW10-60-14		Canola	Wheat	barley
NE20-60-14		Wheat	Canola	wheat
SE13-59-18	50% AY - Echinacea	*Echinacea*	Echinacea	*Summer Fallow*
SW13-59-18		*Canola*	Oats	
Locations near Lanigan				
Burr (RM340)				
NE33-34-22				Canola
NW34-34-22	5-10% AY - Canola	Oats		Canola
NE34-34-22	No aster yellows observed	Canola		
NW35-34-22	No aster yellows observed	Canola	Wheat	
SW4-35-22			Canola	Oats
SE 4-35-22			Wheat	
NE32-34-22			Canola	
SW3-35-22		*Mustard*	Oats	

Table 1 (Continued)

Region / Field location	Aster yellows in canola, caraway and echinacea crops in 2000	Year of aster yellows survey		
		2001	2002	2003
Locations near Brooksby				
Willow Creek (RM 458)				
NE13-46-17		*Alfalfa*	*Barley*	Canola
NW22-46-17			*Oats*	Canola
NW19-46-17		Canola	*Wheat*	Barley
NE21-46-17		*Canola*	*Wheat*	Oats
SW15-46-17			*Peas*	Wheat
SE16-46-17	75% AY - Caraway	Caraway	Oats	
NE24-46-17			*Barley*	Canola
NE9-46-17		*Alfalfa*	Canola	
NW10-46-18		Summer fallow	Caraway	Caraway
Melfort (RM 428)				
NE 26-45-18		*Caraway*	Caraway	
Locations near Kelvington				
Sasman (RM 336)				
SE9-36-11		Canola	Barley	Wheat

Table 1 (Continued)

Region Field location	Aster yellows in canola, caraway and echinacea crops in 2000	Year of aster yellows survey		
		2001	2002	2003
NE17-36-11		Canola	Wheat	Summer fallow
SE29-36-11			Canola	Barley
SW29-36-11			Canola	Wheat
Kelvington (RM 366)				
SW17-37-11	No aster yellows observed			Canola
NW18-38-11	No aster yellows observed			Canola
Location near Canora Mikado (RM 273) (15-25% Aster yellows in the Mikado region)				
NE13-30-3		Canola	Canola	
Sec24-29-3		Canola	Wheat	Canola
Sec17-29-3			Canola	Wheat
NE26-29-2		Canola	Wheat	Canola
NW33-29-2		Canola	*Wheat*	Canola
Norquay (RM 303)				
SW3-2-32		Canola		
SE2-32-2		Canola		

Table 1 (Continued)

Region / Field location	Aster yellows in canola, caraway and echinacea crops in 2000	Year of aster yellows survey		
		2001	2002	2003
Kamsack (RM 271)				
SE26-28-30		Canola		
Locations near Vonda Aberdeen (RM 373)				
SW3-39-2		Canola		
SW1-39-2			Wheat	Canola
Grant (RM 372)				
NE31-38-1			Barley	Wheat
Locations near Coderre Rodgers (RM 133)				
SW5-14-3		*Echinacea*	Summer Fallow	
NE31-14-3 (2 acres)			Echinacea	Echinacea
NE 31-14-3			Mustard	wheat
SW14-14-3				Canola
SE16-15-2				Canola
Sec3-14-3				Wheat

Table 1 (Continued)

Region / Field location	Aster yellows in canola, caraway and echinacea crops in 2000	Year of aster yellows survey		
		2001	2002	2003
Sec 35-13-3				Barley
Other sites surveyed occasionally				
Location near Landis				
Wilkie (RM 379)				
Sec 17-37-18			Canola	
Sec 18-37-18			Wheat	
Location near Togo				
Kamsack (RM 271)				
SE 26-28-30		Canola		
SW 27-28-30		Wheat		
Location near Central SK (GPS location)				
Viscount (RM341)				
51.54.997 / 105.28.525		Canola		
51.54.759 / 105.27.122		Canola		
Winyard (RM 308)				
51.45.085 / 104.23.361		Canola		

Table 1 (Continued)

Region / Field location	Aster yellows in canola, caraway and echinacea crops in 2000	Year of aster yellows survey		
		2001	2002	2003
Foam Lake (RM 276)				
51.39.719 / 103.37.108		Canola		
Yorkton (RM 244)				
51.22.657 / 102.49.039		Canola		
Melville Beach (RM184)				
50.43.433 / 102.32.206		Canola		
Abernethy (RM 186)				
50.44.357 / 103.21.615		Canola		
Earl Grey (RM 219)				
50.56.117 / 104.35.026		Canola		
Nokomis (RM 280)				
51.35.242 / 105.00.399				
Regina (RM 159)				
50.36.7 / 104.46.8		Canola		
50.25.6 / 104.24.6		Canola		

Table 2. Leafhopper species that were found in Saskatchewan in the course of the aster yellows survey, 2001-2003 and that have been reported to vector or carry phytoplasmas.

Species	Disease / phytoplasma strain	Plant hosts	Disease location	References
Ceratagalia humilis Kirk. (= *Aceratagalia sanguinolenta*)	Carrier of alfalfa witches' broom	Alfalfa	Canada	Khadhair et al. 1997
Endria inimica Say	Vector of aster yellows phytoplasma	Unknown	USA	Beanland & Wolf 2003
Euscelis maculipennis Delong & Davidson	Vector of aster yellows phytoplasma	Unknown	Canada	Beirne 1957
Gyponana sp.	Carrier of aster yellows phytoplasma	Unknown	USA	Hill & Sinclair 2000
Macrosteles quadrilineatus Forbes (=*Macrosteles fascifrons* Stål)	Vector of clover phyllody (16SrI-C), California aster yellows (16SrI-A), European aster yellows (16SrI-B) and clover proliferation (16SrVI-A) Carrier of alfalfa witches' broom	Potato, lettuce clover, carrot, vegetable crucifers, many other species	Worldwide	Chiykowski & Sinha 1990 Hoy et al. 1992 Khadhair et al. 1997 Lee et al. 1998
Neokolla hieroglyphica Say	Vector of alfalfa witches' broom	Alfalfa	Canada	Khadhair et al. 1997
Psammotettix sp.	Carrier of 16SrI phytoplasma and tomato stolbur	Unknown	Europe	Zreik et al. 1990 Marzachi et al. 1998
Scaphytopius acutus Say	Vector of western X disease (16SrIII-A) and California aster yellows (16SrI-A) Carrier of *Prunus* and peach X disease and aster yellows phytoplasma	Peach, *Prunus*	USA	Chiykowski & Sinha 1990 Lee et al. 1998 Hill & Sinclair 2000

Table 3. Percent infected leafhoppers at all sites surveyed, 2001-2003.

Species	2001			2002			2003		
	No. of	No. of infecte	%Infecte d	No. of	No. of infecte	%Infecte d	No. of	No. of infecte	%Infecte d

	tested insects	d insects	insects[1]	tested insects	d insects	insects[1]	tested insects	d insects	insects[1]
*Macrosteles quadrilineatus**	3,625	305	8.4%	1,752	78	4.4%	2,228	43	1.9%
*Ceratagalia humilis**	61	6	9.8%	282	1	0.3%	335	6	1.8%
Diplocolenus configuratus	3	1		27	1		16	2	
*Endria inimica**	50	2	4.0%	61	1	1.6%	99	1	1.0%
*Euscelis maculipennis**	10	3		2	0		0	0	
Gyponana sp.*	2	1		0	0		0	0	
*Neokolla hieroglyphica**	3	1		0	0		0	0	
Psammotettix sp.*	1	0		60	2	3.3%	25	0	
*Scaphytopius acutus**	4	1		1	1		0	0	
Sorhoanus ulheri	0	0		1	1		16	0	

[1] Percentage values were calculated only for species where the number of insects tested was ≥50.

*Leafhopper species known to be phytoplasma vectors or carriers.

Table 4. Number of adult leafhoppers and adults of other species of hemiptera caught in emergence traps at all locations near Medstead and Brooksby and at the Saskatoon site, 2001-2003.

Species	Medstead		Brooksby	Saskatoon	
	2001	2003	2002	2002	2003
*Macrosteles quadrilineatus**				2	4
*Ceratagalia humilis**	7	5	3		4
Cicadulla sp.	1				
Dorycephalus platyrhynchus			1		
Empoasca sp.	1				
Erythroneura sp.				1	
Helochora communis		1			
*Neokolla hieroglyphica**	1				
Psammotettix sp.*		1			1
Other species of hemiptera:					
Delphacids (plant hoppers)	2				
Cercopids (spittlebugs)	1				

*Leafhopper species known to be phytoplasma vectors or carriers.

Table 5. Number of leafhoppers caught with sweeping nets on the edge of canola fields (20 sweeps per field) and inside fields (20 sweeps at 5, 10, 20 and 50 m inside the fields), for all fields visited in 2001.

	Medstead 2 fields 15 visits per field		North Battleford 4 fields 5 visits per field		Turtleford 4 fields 5 visits per field		Meadow Lake 3 fields 5 visits per field		Lanigan 2 fields 12 visits per field		Brooksby 1 field 13 visits		Kelvington 2 fields 5 visits per field		Canora 8 fields 8 visits per field		Vonda 1 field 6 visits		Others: Central SK 12 fields 5 visits per field	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In
*Macrosteles quadrilineatus**	129	28	2	1	28	28	15	31	6	13	169	119	13	16	168	157	35	1	42	140
*Ceratagalia humilis**	1										3				6		3	1	9	
Diplocolenus configuratus	34		21	1	3	1	8						1		8				70	1
*Endria inimica**	53	1	11		8	0	30		31	2	15		18	2	79	20	16		156	11
*Euscelis maculipennis**											10									
Gyponana sp.*	1																			
Psammotettix sp.*	40	1			1		48		2						10				8	4
*Scaphytopius acutus**	8	1	1				3													
Sorhoanus ulheri	30		4		5		6								6		1		26	
Agallia quadripunctata							3	0												

Table 5 (Continued)

	Medstead 2 fields, 15 visits per field		North Battleford 4 fields 5 visits per field		Turtleford 4 fields 5 visits per field		Meadow Lake 3 fields 5 visits per field		Lanigan 2 fields 12 visits per field		Brooksby 1 field 13 visits		Kelvington 2 fields 5 visits per field		Canora 8 fields 8 visits per field		Vonda 1 field 6 visits		Others: Central SK 12 fields 5 visits per field	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In
Agalliopsis	2	0									1	0								

sp.																				
Athysanella sp.	3	0													1	0	1		1	0
Athysanus argentarius	1	0	1						3	0	10	0	2	0	48	0	18		743	3
Auridius auratus							1	0												
Auridius ordinatus	2	0																		
Ballana sp.	1	0																		
Balclutha impicta	47	0			2	1			11	2	2	2	0	1	8	3			13	2
B. neglecta	18	0					0	1					1	0	5	0			33	20
Chlorotettix unicolor	1	0	6		3										11	1			4	0
Cicadula sp.	60	0			0	1	2	3	1	1	5	0			4	2	1		3	0
Comellus comma	1	0																		
Cosmotettix bilineatus																			2	0
Cuerna striata	7	0															2		2	0
Deltocephalus sp.	1	0																		

Table 5 (Continued)

	Medstead 2 fields 15 visits per field		North Battleford 4 fields 5 visits per field		Turtleford 4 fields 5 visits per field		Meadow Lake 3 fields 5 visits per field		Lanigan 2 fields, 12 visits per field		Brooksby 1 field 13 visits		Kelvington 2 fields 5 visits per field		Canora 8 fields 8 visits per field		Vonda 1 field 6 visits		Others: Central SK 12 fields 5 visits per field	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In
Dicraneura carneolata	1	0	1		2		3	0			1	0			2	0	5		70	0
Doratura stylata	179	1			2		61	2			1	0			45	0			16	0
Draeculacephala sp.	6	0			1		1	1	1	0					12	0				
Elymana virescens	10	0			6															

Empoasca sp.	0	1			1				1	0			1	0	3	0			1	0
Erythroneura sp.	1	0																		
Errastunus ocellaris					2										2	0			1	0
Euscelis sp.											6									
Flexamia sp.									6	0					1	0				
Hebecephalus sp.											2	0			2	0			4	0
Hecalus sp.					1														2	0
Idiocerus alternatus	2	0																		
I. populi					1															
Latalus personatus	33	0	4		16				6	0	8	0	8	0	54	5	1		111	0

Table 5 (Continued)

	Medstead 2 fields 15 visits per field		North Battleford 4 fields 5 visits per field		Turtleford 4 fields 5 visits per field		Meadow Lake 3 fields 5 visits per field		Lanigan 2 fields 12 visits per field		Brooksby 1 field 13 visits		Kelvington 2 fields 5 visits per field		Canora 8 fields 8 visits per field		Vonda 1 field 6 visits		Others: Central SK 12 fields 5 visits per field	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In
Limottetix sp.																			1	0
Paluda gladiola	9	0																	2	0
Palus deletor	1	0													1	0				
Parabolocratus sp.	1	0																		
Paraphlesius apertus	0		1																	
Scleroracus paludosus	3		0																	
Sorhoanus orientalis						7														
Strangania rufoscutellata				1																
Strentanus sp.	2		0																1	
Typhlocibinae																			4	

Verdanus evansi	329	1	19		44	3	27	0	3	0	10	0	1	0	156	(
Delphacids (planthoppers)			1													
Membracids (treehoppers)																

*Leafhopper species known to be phytoplasma vectors or carriers.

Table 6. Number of leafhoppers caught with sweeping nets on the edge of canola fields (20 sweeps per field) and inside fields (20 sweeps at 5, 10, 20 and 50 m inside the fields), for all fields visited in 2002.

	Medstead 3 fields 10 visits per field		North Battleford 2 fields 10 visits per field		Turtleford 4 fields 7 visits per field		Meadow Lake 2 fields 5 visits per field		Lanigan 2 fields 5 visits per field		Brooksby 1 field 10 visits		Kelvington 2 fields 5 visits per field		Canora 2 fields 5 visits per field		Coderre 1 field 7 visits		Others: Landis 1 field 6 visits	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In
*Macrosteles quadrilineatus**	54	29	29	51	6	20	28	56	15	32	5	30	1	26	16	54	1	7	1	61
*Ceratagalia humilis**	6	2	1				4	0	12	1	0	1	4	1	6	0	8	55	4	0
*Endria inimica**	8	1	11	2	2		1	0	7	1	1	0	10	0	1	0	2			
Diplocolenus configuratus	11		4		3		1	0	4	0			10	0					3	0
*Euscelis maculipennis**				2																
Gyponana sp.*																				
Psammotettix sp.*	14	5	13	1	1	1	18		7	0			3	0	2	0			2	0
*Scaphytopius acutus**					1	1		1												
Sorhoanus ulheri					3		27				1	0	5	0					1	0
Agalliopsis sp.	11	1																		
Athysanus argentarius			3		3				1	0			31	0	11	0				
Auridius auratus			1		1														6	0

Table 6 (Continued)

	Medstead 3 fields 10 visits per field		North Battleford 2 fields 10 visits per field		Turtleford 4 fields 7 visits per field		Meadow Lake 2 fields 5 visits per field		Lanigan 2 fields 5 visits per field		Brooksby 1 field 10 visits		Kelvington 2 fields 5 visits per field		Canora 2 fields 5 visits pe field	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	I

Balclutha impicta	24	1	3		7	1	3	0	3	0	6	1	5	0	1	
B. neglecta	2	1	1	1									1	0		
Chlorotettix unicolor									2	0			3	0		
Cicadula sp.		1						1								
Colladonus torneellus						1										
Commellus sexvitatus		1														

Cuerna striata	17												1	0		
Dicraneura carneolata					1		3	0	9	0			4	0		
Doratura stylata	10				1		36	0	1	0	1	0	44	0	1	
Draeculacephala sp.	14															
Elymana virescens		1					1	0								
Empoasca sp.	5						1				1	0	0	1		
Erythroneura sp.	1															

Table 6 (Continued)

	Medstead 3 fields 10 visits per field		North Battleford 2 fields 10 visits per field		Turtleford 4 fields 7 visits per field		Meadow Lake 2 fields 5 visits per field		Lanigan 2 fields 5 visits per field		Brooksby 1 field 10 visits		Kelvington 2 fields 5 visits per field		Canora 2 fields 5 visits pe field	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	I
Errastunus ocellaris	2				1		5								1	
Flexamia sp.			1		1								1	0		
Hebecephalus sp.			2		2											
Idiocerus lachrymalis					1						1	0				
I. populi					2		1									
Laevicephalus sp.	2				1											
Latalus personatus	23		16		28		5		4	0			17	0		
Limottetix sp.																
Palus bilineatus			1		1											
Paluda gladiola	3															
Parabolocratus sp.						1			4	0			2	0		
Rosenus sp.	1						1	0								
Scaphytopius sp.	5	1			1	1										
Scleroracus paludosus				1		1		1								

Table 6 (Continued)

	Medstead 3 fields 10 visits per field		North Battleford 2 fields 10 visits per field		Turtleford 4 fields 7 visits per field		Meadow Lake 2 fields 5 visits per field		Lanigan 2 fields 5 visits per field		Brooksby 1 field 10 visits		Kelvington 2 fields 5 visits per field		Canora 2 fields 5 visits pe field	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	I
Sorhoanus orientalis							1									
S. ulheri	16	1														
Typhlocibinae	4		4		2		3		1	0	1	0	6	0	2	
Verdanus evansi	126	2	12		3		18		18	0	1	0	63	0	2	

Delphacids (planthoppers)	4		3		1		3		1				2		3	
Fulgorids (planthoppers)	9			1	2											
Membracids (treehoppers)	41		1		8				9		1		17		3	
Cercopids (spittlebugs)	6				1	2	1						1			

*Leafhopper species known to be phytoplasma vectors or carriers.

Table 7. Number of leafhoppers caught with sweeping nets on the edge of canola fields (20 sweeps per field) and inside fields (20 sweeps at 5, 10, 20 and 50 m inside the fields), for all fields visited in 2003.

	Medstead 2 fields 6 visits per field		Turtleford 4 fields 7 visits per field		Meadow Lake 2 fields 5 visits per field		Lanigan 2 fields 7 visits per field		Brooksby 2 fields 8 visits per field		Kelvington 2 fields 6 visits per field		Canora 3 fields 5 visits per field		Vonda 1 field 6 visits	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	I
*Macrosteles quadrilineatus**	20	46	25	42	10	295	10	27	43	144	49	34	11	12		
*Ceratagalia humilis**			2				2		3		2		62			
*Endria inimica**	1		1				11	2	10	2	4		31	2		
Diplocolenus configuratus	1		1				1				1					
*Euscelis maculipennis**									2							
Gyponana sp.*																
Psammotettix sp.*		2	2		5		1			1	2		3	1		
*Scaphytopius acutus**																
Sorhoanus ulheri	1		1		19						2					
Athysanus argentarius									4		35		25			
Auridius auratus					4											
Ballana sp.					3											

Table 7 (Continued)

	Medstead 2 fields 6 visits per field		Turtleford 4 fields 7 visits per field		Meadow Lake 2 fields 5 visits per field		Lanigan 2 fields 7 visits per field		Brooksby 2 fields 8 visits per field		Kelvington 2 fields 6 visits per field		Canora 3 fields 5 visits per field		Vonda 1 field 6 visits	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	I
Balclutha impicta	4	12	6	7			8		3	2	10		2		2	
B. neglecta			5							1			3			

Chlorotettix unicolor					1						13		5			
Cicadula sp.																
Dicraneura carneolata											1		23			
Doratura stylata	2	1			3				1		8		23	1		
Elymana acrita																
Elymana virescens													2			
Errastunus ocellaris													1			
Flexamia sp.																
Hebecephalus sp.									1				1			
Hecalus sp.		2	1						1				1			
Latalus personatus					1		14				9		29			
Palus bilineatus										1	2					

Table 7 (Continued)

	Medstead 2 fields 6 visits per field		Turtleford 4 fields 7 visits per field		Meadow Lake 2 fields 5 visits per field		Lanigan 2 fields 7 visits per field		Brooksby 2 fields 8 visits per field		Kelvington 2 fields 6 visits per field		Canora 3 fields 5 visits per field		Vonda 1 field 6 visits		Coderre 2 fields 5 visits per field		Prince Albert 2 fields 6 visits per field	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In
Sorhoanus flavovirens			1																	
Typhlocibinae					2		2				2		7						1	
Verdanus evansi		2	6		17				4						1		1		3	
Delphacids (planthoppers)			1	1	1			1	1	1	10						2		1	
Membracids (treehoppers)											3		5						6	
Cercopids (spittlebugs)							2						2				1		1	

*Leafhopper species known to be phytoplasma vectors or carriers.

Table 8. Leafhopper species that tested positive for the presence of phytoplasma DNA in samples collected in canola fields, 2001-2003.

Species	2001 34 fields 236 visits	2002 17 fields 114 visits	2003 21 fields 129 visits	Total 72 fields 479 visits	Per 100 visits		
					2001	2002	2003
*Macrosteles quadrilineatus**	1,105	514	849	2,468	468	450	658
*Ceratagalia humilis**	19	42	72	133	8	36	55
Diplocolenus configuratus	148	36	5	189	62	129	<1
*Endria inimica**	434	58	71	563	183	50	55
*Euscelis maculipennis**	10	2	2	14	4	<1	<1
Gyponana sp.*	1	0	0	1	< 1	0	0
Psammotettix sp.*	118	67	24	209	50	58	18
*Scaphytopius acutus**	13	1	0	14	5	11	0
Sorhoanus ulheri	77	37	23	137	32	67	17

*Leafhopper species known to be phytoplasma carriers or vectors.

Table 9. Average number of leafhoppers trapped per ten visits in various regions in Saskatchewan, 2001-2003, and that tested positive for phytoplasma DNA.

Region		*Macrosteles quadrilineatus**	*Ceratagallia humilis**	*Endria inimica**	*Diplocolenus configuratus*	*Euscelis maculipennis**	*Gyponana* sp.*	*Psammotettix* sp.*	*Scaphytopius acutus**	*Sorhoanus ulheri*
Medstead	2001	52	<1	18	11	0	<1	13	3	10
	2002	83	2	3	4	0	0	6	0	0
	2003	55	0	<1	<1	0	0	1	0	0
Turtleford	2001	30	0	8	13	0	0	2	0	5
	2002	37	<1	10	2	0	0	6	1	1
	2003	55	1	<1	<1	0	0	1	0	0
North Battleford	2001	2	0	6	11	0	0	0	1	2
	2002	40	<1	6	2	2	0	7	0	0
Meadow Lake	2001	30	0	20	5	0	0	32	2	4
	2002	84	4	1	1	0	0	18	1	27
	2003	305	0	0	0	0	0	5	0	19
Lanigan	2001	8	0	13	0	0	0	<1	0	0
	2002	47	13	8	4	0	0	7	0	0
	2003	26	1	8	0	1	0	<1	0	1
Brooksby	2001	221	3	11	0	7	0	0	0	0
	2002	35	1	1	0	0	0	0	0	1
	200	152	1	7	0	1	0	<1	0	1

	3									
Kelvington	2001	29	0	20	1	0	0	0	0	0
	2002	27	5	10	10	0	0	3	0	5
	2003	69	1	3	<1	1	0	1	0	1

Table 9 (continued)

		*Macrosteles quadrilineatus**	*Ceratagalia humilis**	*Endria inimica**	*Diplocolenus configuratus*	*Euscelis maculipennis**	*Gyponana* sp.*	*Psammotettix* sp.*	*Scaphytopius acutus**	*Sorhoanus ulheri*
Canora	2001	50	1	15	1	0	0	1	0	1
	2002	70	6	1	0	0	0	2	0	0
	2003	14	41	22	0	0	0	3	0	1
Vonda	2001	60	7	26	0	0	0	0	0	2
	2003	10	0	0	0	0	0	0	0	0
Coderre	2002	11	90	0	0	0	0	0	0	0
	2003	30	0	7	1	0	0	5	0	0
Landis	2002	99	6	0	5	0	0	3	0	1
Prince Albert	2003	42	<1	0	0	0	0	1	0	0
Central SK	2001	30	1	27	12	0	0	2	0	4

*Leafhopper species known to be phytoplasma carriers or vectors.

Table 10. Number of leafhoppers caught in sweeping nets on the edge of cereal fields (20 sweeps per field) and inside fields (20 sweeps at 5, 10, 20 and 50 m inside the fields), for all fields visited in 2001.

	Medstead 1 field 15 visits		Meadow Lake 4 fields 3 visits per field		Lanigan 1 field 7 visits		Canora 1 field 3 visits	
Species	Edge	In	Edge	In	Edge	In	Edge	In
*Macrosteles quadrilineatus**	243	335	34	666	7	104	11	18
*Ceratagalia humilis**	1				1	2		
*Endria inimica**	5	2	10	17	8	25		
Diplocolenus configuratus	2		6	1	1	1		
*Euscelis maculipennis**				1				
Gyponana sp.*								
Psammotettix sp.*	5	6	9	29				1
*Scaphytopius acutus**			11					
Sorhoanus ulheri	8	2	26					1
Agallia quadripunctata			1					
Agalliopsis sp.			3					
Athysanus argentarius						1		1
Auridius auratus		1		2				
Balclutha sp.	58	6	1	5	6	17		1
B. neglecta	5	15	4					
Chlorotettix unicolor	6		2					1
Cicadula sp.	45	10	1	4	4			
Cuerna striata	16							
Dicraneura carneolata		4						
Doratura stylata		1	6	1				
Draeculacephala sp.	6		4					

Elymana virescens	5		1	6				

Table 10 (Continued)

	Medstead 1 field 15 visits		Meadow Lake 4 fields 3 visits per field		Lanigan 1 field 7 visits		Canora 1 field 3 visits	
Species	Edge	In	Edge	In	Edge	In	Edge	In
Empoasca sp.	13							
Errastunus ocellaris			2					
Flexamia sp.					3			
Hecalus sp.				1				
Idiocerus alternatus			1	1				
I. populi								
Latalus personatus	14		14	4		1		
Limottetix sp				1				
Macrosteles variata			3					
Paluda gladiola	3							
Scaphytopius sp.	2							
Scleroracus paludosus				1				
Sorhoanus flavovirens		3						
Streptanus confinis			1	1				
Verdanus evansi	206	7	58	16				
Delphacids (planthoppers)	5	1						

*Leafhopper species known to be phytoplasma carriers or vectors.

Table 11. Number of leafhoppers caught in sweeping nets on the edge of cereal fields (20 sweeps per field) and inside fields (20 sweeps at 5, 10, 20 and 50 m inside the fields), for all fields visited in 2002.

	Medstead 1 field 10 visits		Turtleford 1 field 7 visits		North Battleford 1 field 6 visits		Meadow Lake 3 fields 5 visits per field		Lanigan 1 field 7 visits		Brooksby 2 fields 10 visits per field		Kelvington 1 field 5 visits		Canora 1 field 5 visits		Vonda 1 field 5 visits		Landis 1 field 6 visits		Central SK 3 fields 1 visit per field	
Species	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In
*Macrosteles quadrilineatus**	11	124	6	20	3	33	44	308	3	59	2	3	31	572	13	198	14	74	1	55	8	96
*Ceratagalia humilis**						1		2	2	2			10	1	5	3	1		4	1	1	1
*Endria inimica**	1	1	2		2	11	13	23		3				2	2	2	3	2		1		3
Diplocolenus configuratus	7		3		7		17	3	20	1			1					1	3			
*Euscelis maculipennis**					2	9								1								
Gyponana sp.*	1																					
Psammotettix sp.*		16	1	1		5	17	64	3	26		2	1	2	21	5	10	45	2	5		6
*Scaphytopius acutus**			1	1																		
Sorhoanus ulheri	1		3		2				1				7					1	1	1		
Agalliopsis sp.	1	1											1									
Agallia quadripunctata													1									
Athysanus argentarius					4				7				14	1	21	1		1				
Athysanus attenuata															2							
Auridius auratus									1										6	3		

Table 11 (Continued)

	Medstead 1 field 10 visits		Turtleford 1 field 7 visits		North Battleford 1 field 6 visits		Meadow Lake 3 fields 5 visits per field		Lanigan 1 field 7 visits		Brooksby 2 fields 10 visits per field		Kelvington 1 field 5 visits		Canora 1 field 5 visits		Vonda 1 field 5 visits		Landis 1 field 6 visits		Central SK 3 fields 1 visit per field	
Species	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In
Balclutha sp.	9	18	7	1	1		7	4				3	9	2	4	1	3	2			3	13
B. neglecta		2			1				1				5					2		2		
Chlorotettix unicolor													1		1							
Cicadula sp.															1							
Colladonus torneellus				1																		
Commellus sexvitatus	4																					
Cuerna sp.	2																					
Dicraneura carneolata		3	1		1		8	5	1				2	1	1	2						
Doratura stylata	14		1				27	1					4		2							
Dorycephalus phurynchus																			1			
Draeculacephala sp.																						1
Elymana acrita		1								1												
E. virescens							2	2														
Empoasca sp.															1							
Endria rotunda									1													

Table 11 (Continued)

	Medstead 1 field 10 visits		Turtleford 1 field 7 visits		North Battleford 1 field 6 visits		Meadow Lake 3 fields 5 visits per field		Lanigan 1 field 7 visits		Brooksby 2 fields 10 visits per field		Kelvington 1 field 5 visits		Canora 1 field 5 visits		Vonda 1 field 5 visits		Landis 1 field 6 visits		Central SK 3 fields 1 visit per field	
Species	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In
Errastunus ocellaris			1													1						
Flexamia sp.			1																			
Hardya dentata							1															
Hebecephalus sp.								1										1				1
Hecalus sp.	3																					
Idiocerus populi			1		1																	
Laevicephalus sp.			1																			
Latalus personatus	3		28		20		15		3			1	4		3				1	1	5	
Palus sp.								6						2			1					
Palus deletor						1																
Parabolocratus sp.				1			1	2							1				2			
Pinumius aeratus								1														
Rosenus sp.							1	4														
Sorhoanus sp.	6																					2
Sorhoanus orientalis		1					14	9														
Typhlocibinae			2		2		18	5			1		9	1	1							
Verdanus evansi	198	8	3		3		101	111	2			1	30	2	20				3			1

**Leafhopper species known to be phytoplasma carriers or vectors.

'. Number of leafhoppers caught in sweeping nets on the edge of cereal fields (20 sweeps per field) and inside fields (20 at 5, 10, 20 and 50 m inside the fields), for all fields visited in 2003.

	Medstead 2 fields 6 visits per field		Turtleford 2 fields 6 visits per field		Meadow Lake 2 fields 2 visits per field		Lanigan 2 fields 6 visits per field		Brooksby 2 fields 6 visits per field		Kelvington 2 fields 5 visits per field		Canora 1 field 5 visits		Vonda 1 field 4 visits	
Species	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	I
*Macrosteles quadrilineatus**	35	89	16	293	21	254	5	235	22	153	7	244		29	2	2
*Ceratagalia humilis**	1		1						4				3			
*Endria inimica**				1		13	31	8			9	4		2		
Diplocolenus configuratus	2	1	2			1	3				3		7			
*Euscelis maculipennis**											1					
Psammotettix sp.*	1	2		1	7	18	4	11			4	1	3	2		
Sorhoanus ulheri	2		2	2	6	2	5		3		3				2	
Athysanus argentarius							58				62		5			
Auridius auratus																
A. ordinatus						1										
Balclutha sp.	8	7	4	6	5	9	7	10	4		22	10	5	1		
B. neglecta	3	3	1	19		3		1	2		1	4		5		
Chlorotettix unicolor							3				9					
Cuerna sp.																

Table 12 (Continued)

	Medstead 2 fields 6 visits per field		Turtleford 2 fields 6 visits per field		Meadow Lake 2 fields 2 visits per field		Lanigan 2 fields 6 visits per field		Brooksby 2 fields 6 visits per field		Kelvington 2 fields 5 visits per field		Canora 1 field 5 visits		Vonda 1 field 4 visits		Coderre 2 fields 3 visits per field		Prince Albert 2 fields 5 visits per field	
Species	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In	Ed	In
Dicraneura carneolata											1									
Doratura stylata	3					1	1				42	2	7							
Elymana acrita					1															
Erythroneura sp.									1											
Hebecephalus sp.		1																		
Latalus personatus	1		3				1		1		13	1	3							
Orocastus perpusillus					1															
Palus bilineatus				1		1									1					
Scaphytopius sp.						1														
Typhlocibinae			3		1	2		1			4	1								4
Verdanus evansi	8						6		2		44	2	2			4	1			
Delphacids (planthoppers)	3		4	5	14	3	1	4			3	4		15				1		5
Fulgorids (planthoppers)		1																		
Membracids (treehoppers)	1		3				1		3		18		1							
Cercopids (spittlebugs)			1				1				2									

*Leafhopper species known to be phytoplasma carriers or vectors.

Table 13. Leafhopper species that tested positive for the presence of phytoplasma DNA in samples collected in cereal fields, 2001-2003.

Species	2001 7 fields 37 visits	2002 16 fields 89 visits	2003 18 fields 87 visits	Total 41 fields 213 visits	Per 100 visits		
					2001	2002	2003
*Macrosteles quadrilineatus**	1,418	1,678	1,581	4,677	3,832	1,885	1817
*Ceratagalia humilis**	4	34	13	51	11	38	15
*Endria inimica**	67	71	71	209	181	80	82
Diplocolenus configuratus	11	63	19	104	30	21	22
*Euscelis maculipennis**	1	12	1	14	1	11	1
Gyponana sp.*	0	1	0	1	0	0	0
Psammotettix sp.*	50	232	62	344	135	69	71
*Scaphytopius acutus**	11	2	0	13	30	12	0
Sorhoanus ulheri	37	17	31	85	100	41	35

*Leafhopper species known to be phytoplasma carriers or vectors.

Table 14. Number of leafhoppers caught in sweeping nets at the edge of herb and spice fields (20 sweeps per field) and inside fields (20 sweeps at 5, 10, 20 and 50 m inside the fields) for all fields visited, 2001-2003.

	2001		2002								2003	
	Brooksby 1 caraway field 13 visits		Brooksby 2 caraway fields 10 visits per field		Coderre 2 echinacea fields 6 visits per field		Landis 1 echinacea field 6 visits		Meadow Lake 1 echinacea field 5 visits		Coderre 1 echinacea field 4 visits	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In
*Macrosteles fascifrons**	49	266	20	61	8	3	1		0	10	1	2
*Ceratagalia humilis**	3	80	1	3	8	94	4	31	0	27	5	19
Diplocolenus configuratus	5		1		4		3	2				
*Endria inimica**	6	1	1	5	3				14		2	
*Euscelis maculipennis**		3		1								
Psammotettix sp.*				1	9		2	2	18		1	
*Scaphytopius acutus**												
Sorhoanus ulheri	5						1		8			
Agalliopsis sp.										1		
Athysanella attenuata											2	
Athysanus argentarus	18		3									

Auridius auratus							6	1				
Balclutha sp.		1	2	1	21	1			2		22	
B. neglecta						6					4	

Table 14 (Continued)

	2001		2002								2003	
	Brooksby 1 caraway field 13 visits		Brooksby 2 caraway fields 10 visits per field		Coderre 2 echinacea fields 6 visits per field		Landis 1 echinacea field 6 visits		Meadow Lake 1 echinacea field 5 visits		Coderre 1 echinacea field 4 visits	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In
Chlorotettix sp.	1		1									
Cicadula sp.	6	6										
Cuerna sp.			6									
Dicraneura sp.			1									
Doratura stylata	1								3			
Elymana sp.									1			
Empoasca sp.					1				1			
Errastunus ocellaris									5			
Erythroneura sp.	1											
Euscelis sp.	6											
Flexamia sp.					2							
Hardya					3							

dentata												
Hebecephalus sp.	3				1							
Idiocerus alternatus		2										
I. populi									1			

Table 14 (Continued)

	2001		2002								2003	
	Brooksby 1 caraway field 13 visits		Brooksby 2 caraway fields 10 visits per field		Coderre 2 echinacea fields 6 visits per field		Landis 1 echinacea field 6 visits		Meadow Lake 1 echinacea field 5 visits		Coderre 1 echinacea field 4 visits	
Species	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In	Edge	In
Latalus personatus	12	1					1		5			
Palus sp.					4							
Rosenus sp.									5			
Parabolocratus sp.			3				2					
Sorhoanus sp.									4			
Sorhoanus orientalis					3							
Typhlocibinae	3		2						3		1	
Verdanus evansi	16		3		5		3	3	5			

*Leafhopper species known to be phytoplasma carriers or vectors.

Table 15. Leafhopper species that tested positive for the presence of phytoplasma DNA in samples collected in herb and spice crops, 2001-2003.

Species	2001 1 field 13 visits	2002 5 fields 43 visits	2003 1 field 4 visits	Total 7 fields 60 visits	Per 100 visits		
					2001	2002	2003
*Macrosteles quadrilineatus**	315	103	3	421	2,423	239	75
*Ceratagalia humilis**	83	168	24	275	638	390	600
Diplocolenus configuratus	5	10	0	15	38	23	0
*Endria inimica**	7	23	2	32	53	53	50
*Euscelis maculipennis**	3	1	0	4	23	2	0
Gyponana sp.*	0	32	0	32	0	74	0
Psammotettix sp.*	0	0	1	1	0	0	25
*Scaphytopius acutus**	0	0	0	0	0	0	0
Sorhoanus ulheri	5	9	0	14	38	20	0

*Leafhopper species known to be phytoplasma carriers or vectors.

Macrosteles quadrilineatus caught with sweeping nets in canola and cereal fields, 2001-2003.

	2001		2002		2003	
Crop	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs
Canola	468	5	450	0	658	52
Cereals	3,832	1,594	1,885	1,393	1,817	526

Table 17. Number of adults of *Macrosteles quadrilineatus* caught with sweeping nets at the edge and inside canola and cereal fields per 100 visits and per 20 sweeps, 2001-2003.

		Canola fields	Cereal fields
2001	Edge	257	797
	In	56	7,587
2002	Edge	136	152
	In	80	433
2003	Edge	176	182
	In	121	408

Table 18. Frequency of *Macrosteles quadrilineatus* individuals that tested positive for phytoplasma DNA per region and year. (When the total number of tested insects is <50, the number of positive insects and the number of tested insects are indicated.)

		2001			2002			2003		
Region		Canola	Cereals	Herb / Spice	Canola	Cereals	Herbs / Spices	Canola	Cereals	Herbs / Spices
Medstead	Edge In	2.6% 3.3%	1.8% 12.6%		22% 9.6%	7.1% 16%		0% 0%	0% 0%	
Brooksby	Edge In	0% 0.4%		65% 60%	12% 0%	11% 0%	13.6% 0%	3.1% 1.6%	0% 4.6%	
Canora	Edge In	2.7% 3.7%	3 / 4 7.7%		6% 14%	1 / 4 0.8%		05 0.95	0%	
Kelvington	Edge In	0% 0%	0% 0%		2 / 8 4.1%	1 / 12 0.6%		0% 1 / 10	0% 1.8%	
Lanigan	Edge In	0% 0%	0% 0%		0% 5.8%			1 / 7 1 / 15	0% 2.0%	
Meadow Lake	Edge In	0% 0%	4% 3.8%		5.4% 2.9%	0% 1.8%	37%	0% 1.2%	3.2% 1.2%	
Turtleford	Edge In	0% 0%			0% 0%			2 / 32 1.2%	0 / 14 1.9%	
North Battleford	Edge In	0% 0%			2.1% 0%	1 / 3 2 / 15				
Vonda	Edge In	0% 0%				1 / 10 1.8%		0% 0%	0% 0%	

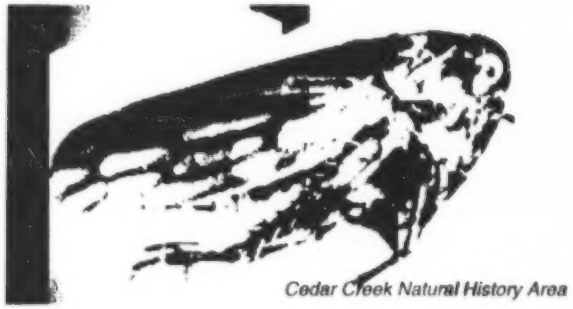
Table 18 (Continued)

		2001			2002			2003		
		Canola	Cereals	Herbs / Spices	Canola	Cereals	Herbs / Spices	Canola	Cereals	Herbs / Spices
Coderre	Edge In				1 / 10 0%	0% 0%	0% 1 / 9	0% 0%	0% 1 / 29	0% 0%
Prince Albert	Edge In							1 / 24 0%	1.1%	
Landis	Edge In				1 / 5 0%	0% 2 / 30				
Saskatchewan survey	Edge In	3.2% 0.6%			0%					

Macrosteles quadrilineatus Forbes

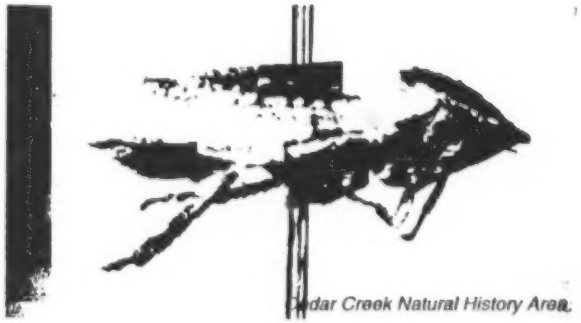
Fig. 1: Biological cycle of the aster leafhopper *Macrosteles quadrilineatus* Forbes, from the egg stage (arrow: eye of the embryo), the nymph stage (5 stages) and adulthood. (bar: 1 mm)

Ceratagalia humilis Kirk.



Endria inimica Say

Gyponana sp.



Neokolla hieroglyphica Say

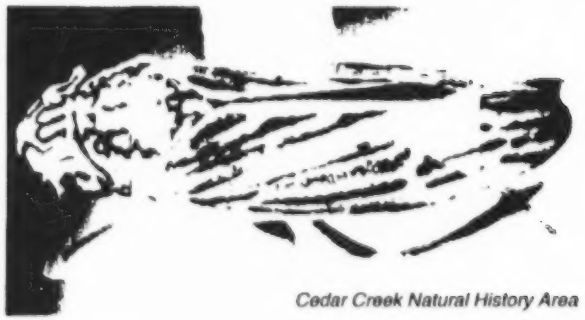
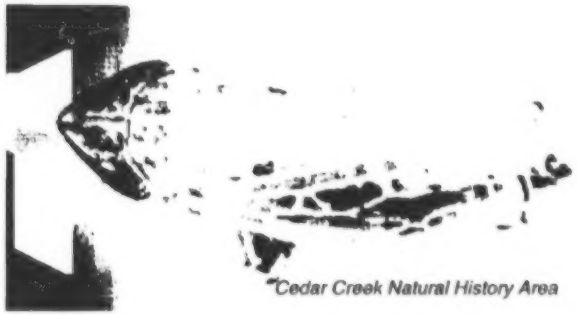
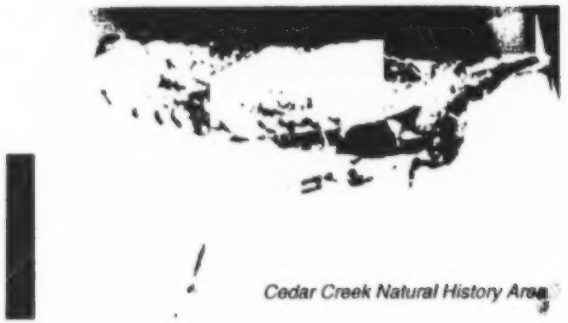


Fig. 2: Leafhopper species that tested positive for phytoplasma DNA.

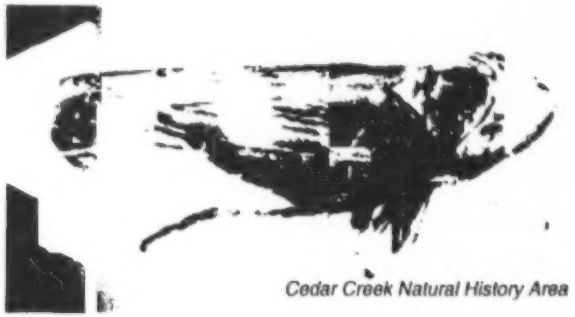
Diplocolenus configuratus Uhler



Scaphytopius acutus Say



Psammotettix sp.



Sorhoanus sp.

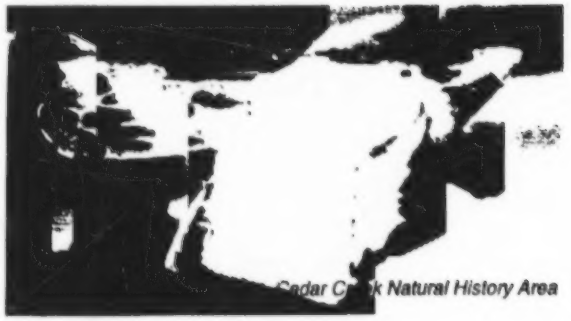


Fig. 3: Leafhopper species that tested positive for phytoplasma DNA.

Fig. 4: Emergence trap set up in a canola field near Medstead, SK.

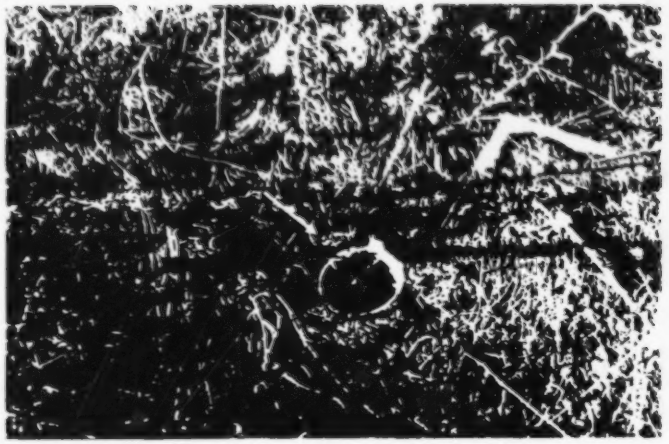


Fig. 5: Pitfall buried in the soil at the edge of a caraway field near Brooksby, SK.

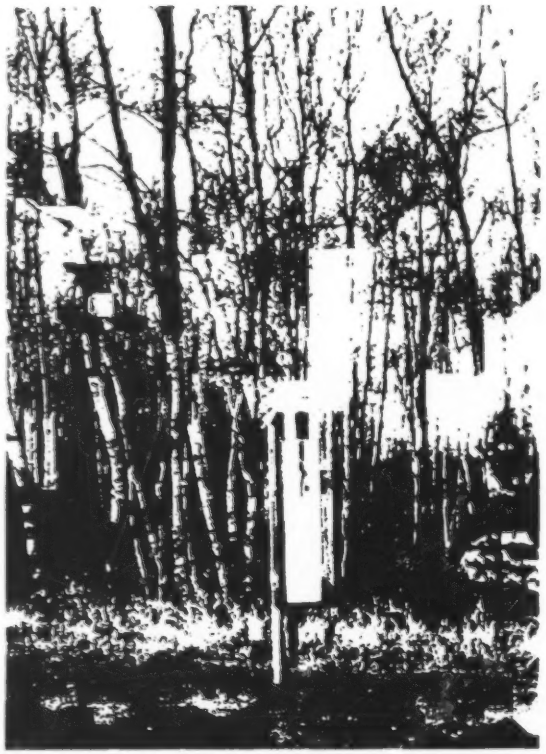


Fig. 6: Suction trap set up at the edge of a glade near Medstead, SK.

Fig. 7: Use of a sweeping net in a flowering canola field near Canora, SK.

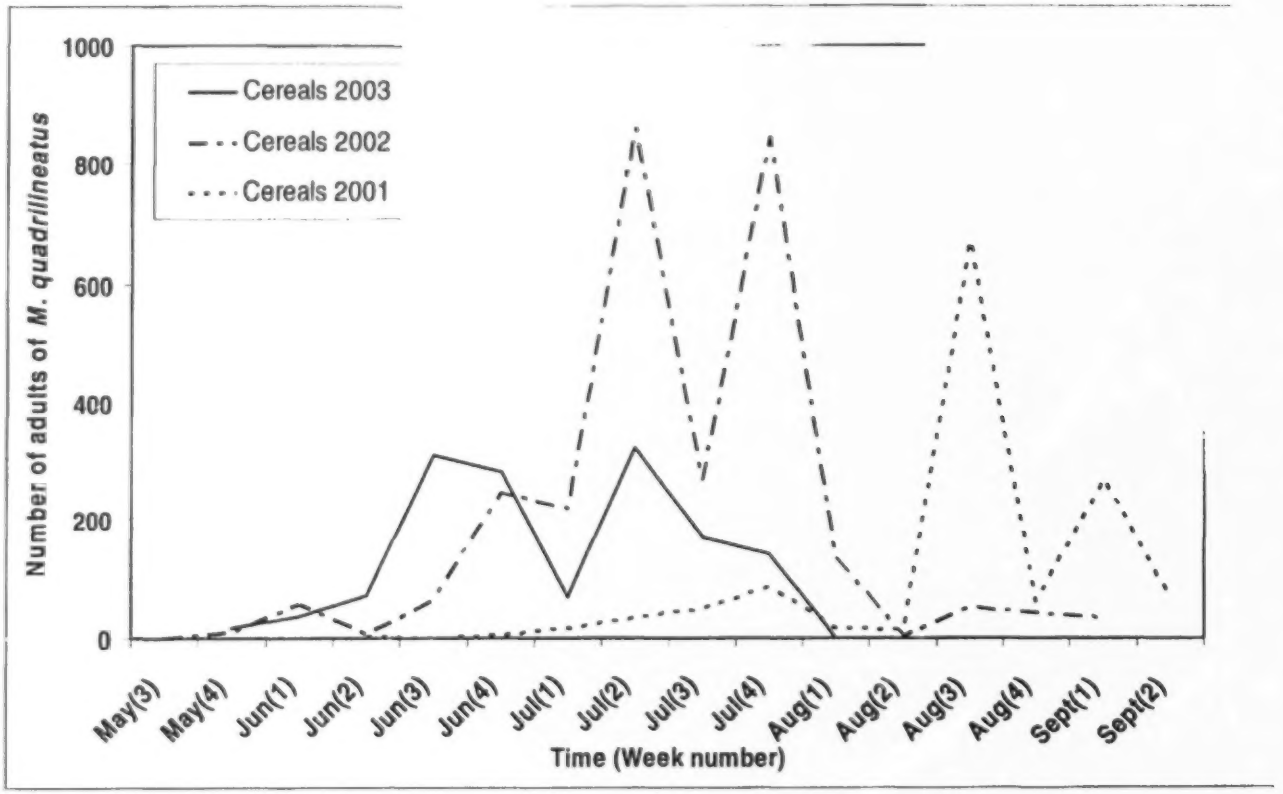


Fig. 8: Evolution of the adult population of *M.quadrilineatus* in cereal fields during the growing seasons in 2001-2003.

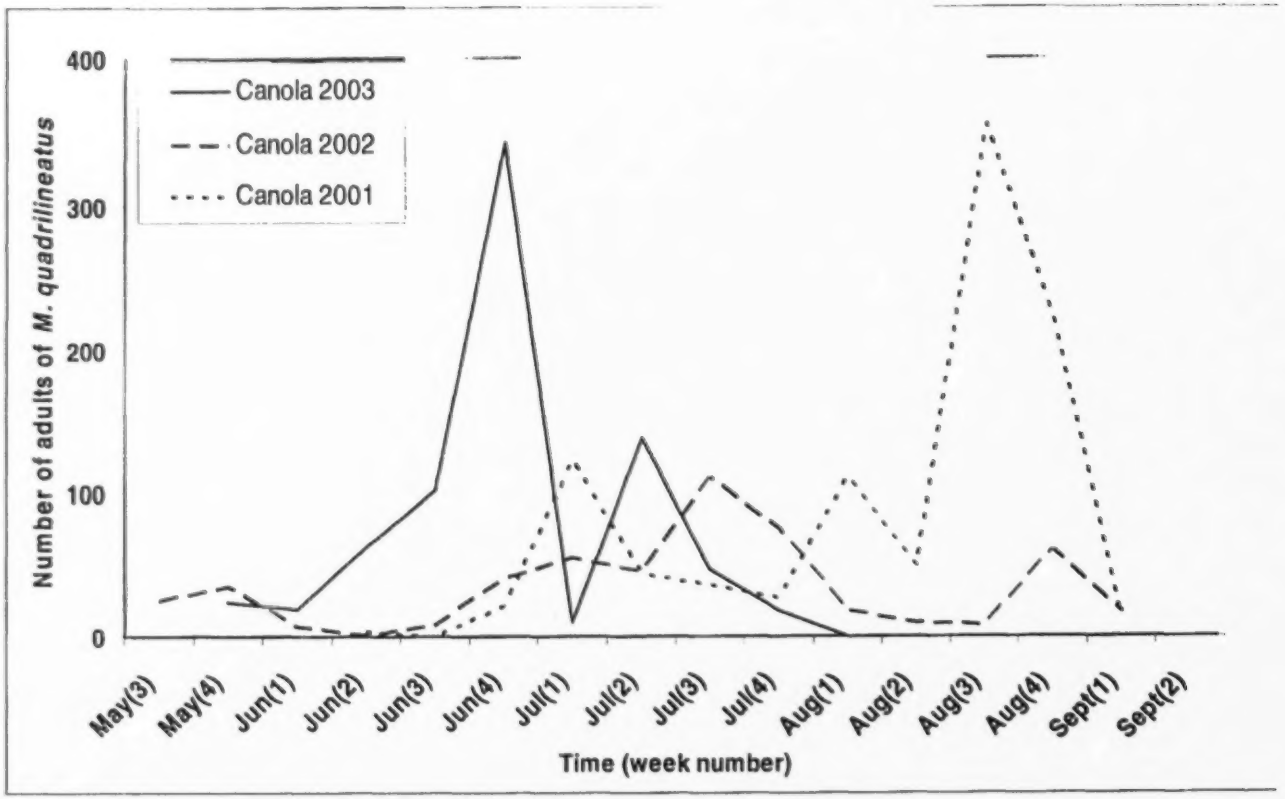


Fig. 9: Evolution of the adult population of *M.quadrilineatus* in canola fields during the growing seasons in 2001-2003.

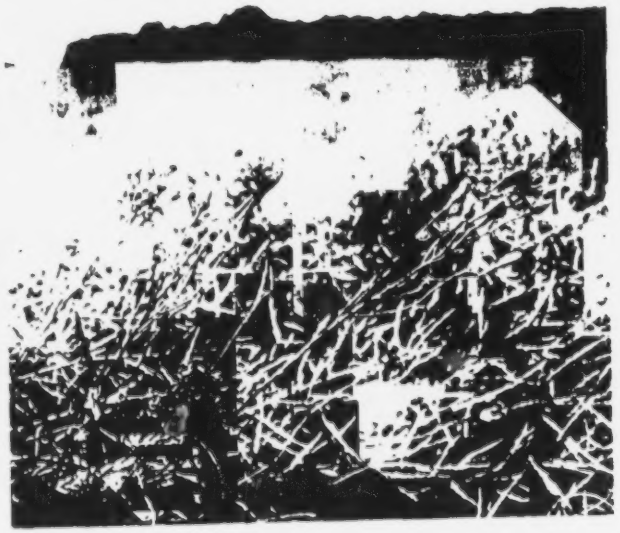


Fig. 10: Canola plant with aster yellows symptoms in a canola field near Canora, SK.

Fig. 11: Close-up of a canola inflorescence with aster yellows symptoms (arrow: bladder-like silique).

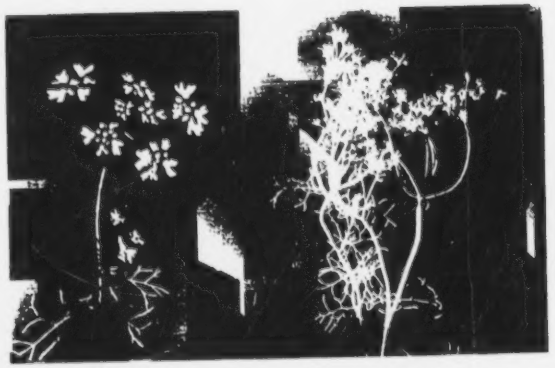


Fig. 12: Caraway plant with aster yellows symptoms (right) and a non-symptomatic plant (left).

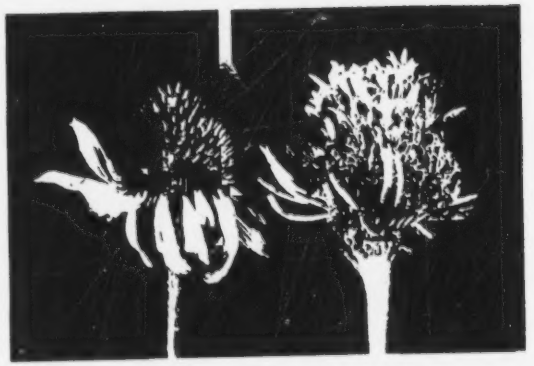


Fig. 13: Echinacea with aster yellows symptoms (right) and a non-symptomatic flower (left).

Fig. 14: Hawk's beard plant with aster yellows symptoms (right) and a non-symptomatic plant (left).

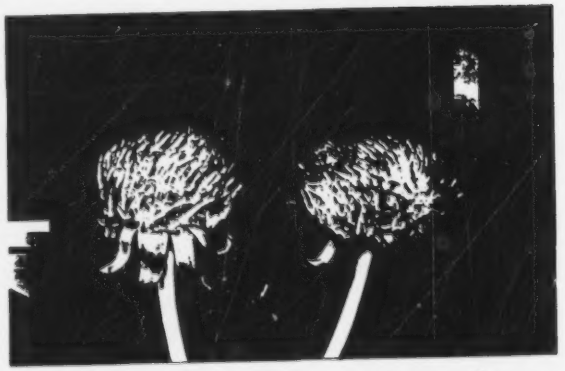


Fig. 15: Dandelion flowers with aster yellows symptoms.

Fig. 16: Raspberry plant with aster yellows symptoms near Medstead, SK.

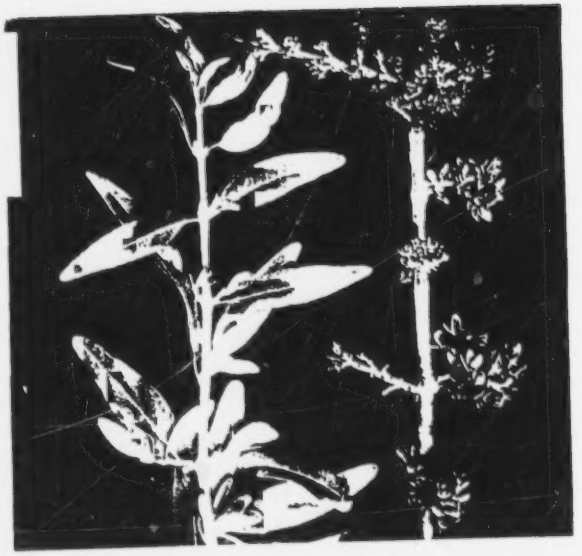


Fig. 17: Sea-buckthorn plant with aster yellows symptoms (right) and a non-symptomatic plant (left).

Fig. 18: Aster yellows symptoms on honeysuckle (right) and non-symptomatic branch from the same tree (left).

Fig. 19: Non-symptomatic barley head (spike at the left) and aster yellows symptoms, depending on the infection time (second from left spike to right).

Fig. 20: Silique from non-symptomatic canola plants containing well-formed seeds (uppermost slilique) and 3 siliques from a plant with aster yellows symptoms, showing well-formed and misshapen seeds.

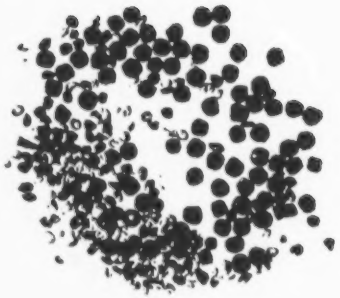


Fig. 21: Well-formed and misshapen seeds from a canola plant with aster yellows symptoms.

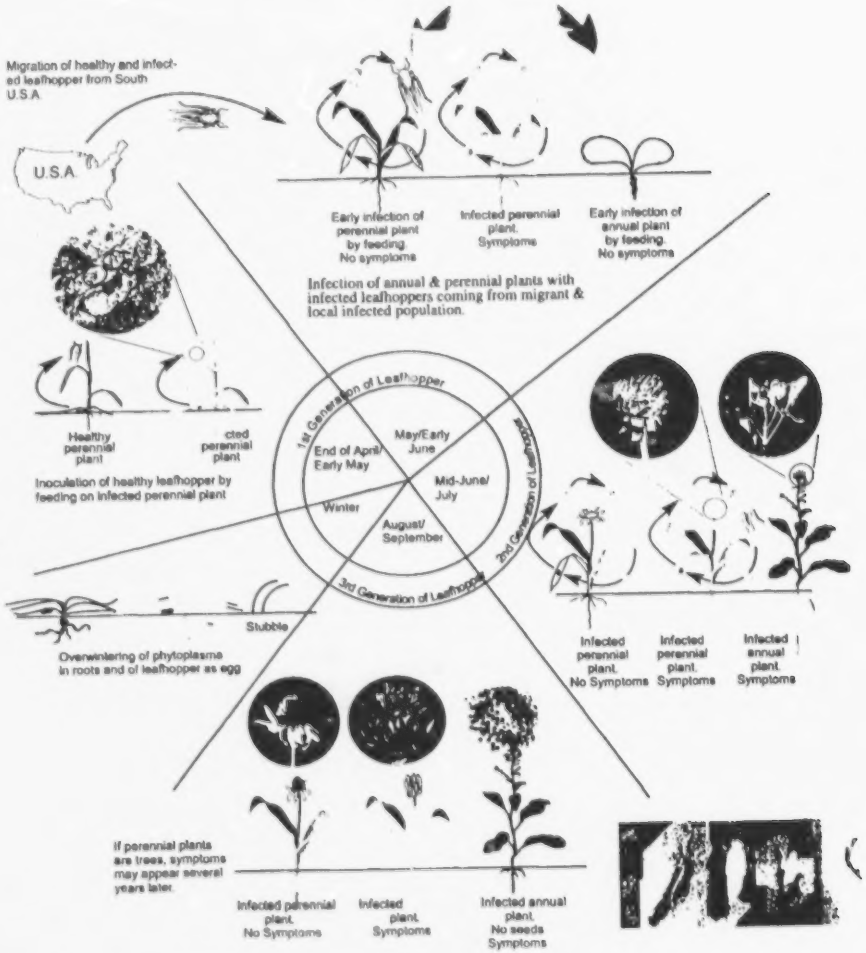

Migration of healthy and infected leafhopper from South U.S.A
U.S.A
Early infection of perennial plant by feeding. No symptoms
Infected perennial plant. Symptoms
Early infection of annual plant by feeding. No symptoms
Infection of annual & perennial plants with infected leafhoppers coming from migrant & local infected population.
Healthy perennial plant
cted perennial plant
Inoculation of healthy leafhopper by feeding on infected perennial plant
1st Generation of Leafhopper
2nd Generation of Leafhopper
3rd Generation of Leafhopper
End of April/ Early May
May/Early June
Mid-June/ July
August/ September
Winter
Stubble
Overwintering of phytoplasma in roots and of leafhopper as egg
Infected perennial plant. No Symptoms
Infected perennial plant. Symptoms
Infected annual plant. Symptoms
If perennial plants are trees, symptoms may appear several years later.
Infected perennial plant. No Symptoms
Infected plant. Symptoms
Infected annual plant. No seeds Symptoms

Fig. 22: Life cycle of the aster yellows disease.

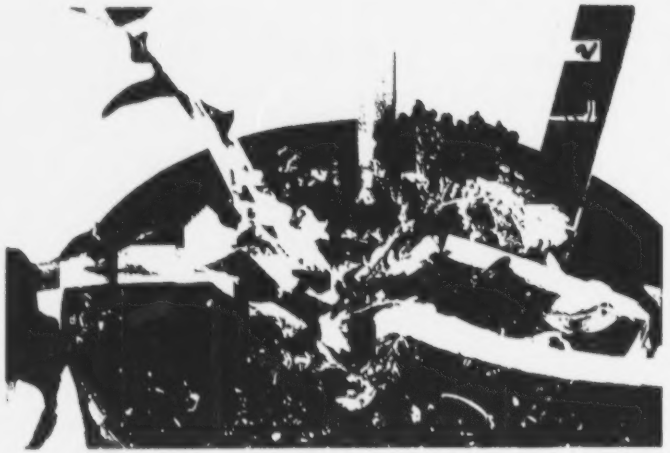


Fig. 23: Canola plant showing no growing point.

Fig. 24: Canola plant showing a stocky and twisted stem.

Fig. 25: Puckered canola leaf with numerous trichomes.

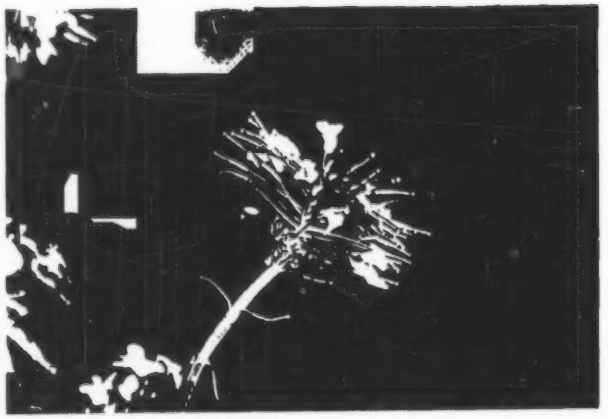


Fig. 26: Compressed inflorescence on canola plant.

Fig. 27: Healthy canola plant (left) and three progeny plants from phytoplasma-infected canola plants showing delayed growth.